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Aerosols Generated by Liquid Sludge Application to Land

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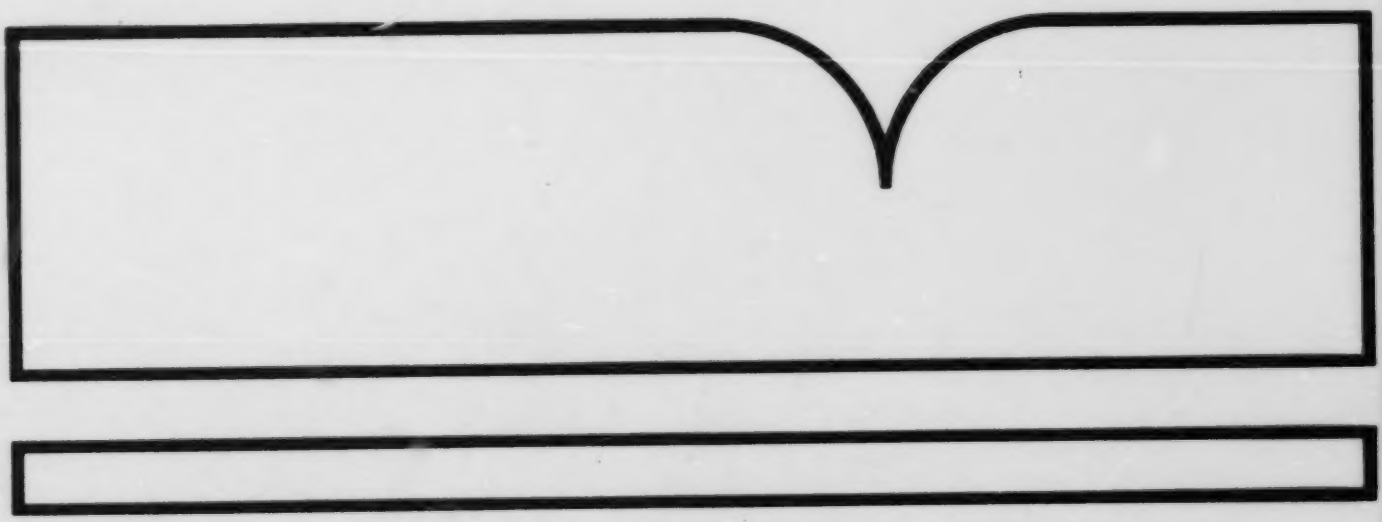

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16. ABSTRACT

A preliminary screen was conducted at six sites to characterize sludge with regard to bacterial and viral microorganisms, trace metals, organochlorine pesticides, and PCB's, and to evaluate each site for its suitability for aerosol monitoring. Four sites were selected for aerosol monitoring, two practicing tank truck application and two practicing spray gun application. From five to eight aerosol monitoring runs were made at each of the four sites, and a special enterovirus aerosol run was conducted at one of the spray sites.

The preliminary screens were used to indicate what levels of microbiological and chemical constituents could be routinely expected in liquid sludge. The aerosol data were used to indicate whether there was aerosolization of microbiological constituents and as input to dispersion modeling.

There was some evidence of aerosolization at the tank truck sites and strong evidence at the spray sites, particularly in the fecal coliform and fecal streptococci data. Modeling of the results proved difficult and there is considerable uncertainty in the data presented. No human enteric viruses were detected on the special run, which translates into a concentration of less than 0.0016 pfu/m^3 at a distance of 40 m downwind from the spray gun. The inability to detect viruses in the air most likely resulted from the low concentration in the sludge and from their adsorption into the solid matter in the sludge which is not easily aerosolized.

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AEROSOLS GENERATED BY LIQUID SLUDGE APPLICATION TO LAND

by

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report provides data on characterization of geographically diverse digested municipal sludges with respect to microbiological, trace metal, pesticide, and polychlorinated biphenyl constituents plus microorganism levels in aerosols generated in the land application of these sludges utilizing tank trucks and spray guns. With a knowledge of aerosol levels from this practice, recommendations can be made to reduce exposure to potentially harmful materials.

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Director
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ABSTRACT

Since previous studies have shown that aerosolized wastewater does transport pathogenic organisms away from the spray site, it can be inferred that there is a potential for similar transport of pathogenic organisms when liquid sludge is land applied in a manner that gives rise to aerosol generation. A monitoring program was conducted at four geographically dispersed sites to study this potential transport.

A preliminary screen was conducted at six sites to characterize sludge with regard to bacterial and viral microorganisms, trace metals, organochlorine pesticides, and polychlorinated biphenyls (PCB's), and to evaluate each site for its suitability for aerosol monitoring. Four sites were selected for aerosol monitoring, two practicing tank truck application and two practicing spray gun application. From five to eight aerosol monitoring runs were made at each of the four sites, and a special enterovirus aerosol run was conducted at one of the spray sites.

The preliminary screens were used to indicate what levels of microbiological and chemical constituents could be routinely expected in liquid sludge. The aerosol data were used to indicate whether there was aerosolization of microbiological constituents and as input to dispersion modeling.

There was some evidence of aerosolization at the tank truck sites and strong evidence at the spray sites, particularly in the fecal coliform and fecal streptococci data. Modeling of the results proved difficult and there is considerable uncertainty in the data presented. No human enteric viruses were detected on the special run, which translates into a concentration of less than 0.0016 pfu/m^3 at a distance of 40 m downwind from the spray gun. The inability to detect viruses in the air most likely resulted from the low concentration in the sludge and from their adsorption onto the solid matter in the sludge which is not easily aerosolized.

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SECTION 1

INTRODUCTION

BACKGROUND

Land application of human waste is an attractive waste disposal alternative to disposal in surface waters. This approach avoids contamination of the surface water supplies, returns nutrients to the soil, reuses the water and other wastes, and provides additional waste treatment. The policy of the EPA is to "press vigorously for publicly-owned treatment works to utilize land treatment processes to reclaim and recycle municipal wastewater" (1). Disposing of municipal sludge has become a major public concern in that sludge will be produced at municipal treatment plants in the U.S. at the rate of approximately four and one-half billion dry kilograms annually by the early 1980's. At the present time, municipal sludge is being disposed of via incineration (35%), via ocean disposal (15%), in landfills (25%), and by land application (25%) (2). Incineration contributes to air pollution and is also energy dependent. Federal regulations will prohibit ocean dumping of sludge after 1981. Landfill is not practical in all areas of the U.S. and has potential problems in contamination of ground and surface waters due to the concentration of sludge in one area. Land application is an attractive alternative to these processes.

An excellent review of the possible health risks associated with the application of municipal sludge to land is found in Pahren et al. (2). Part of the problem in application of sludge to land is the formation of aerosols containing pathogenic bacteria and viruses. The possibility of health risks from public and occupational exposures to these types of aerosols was recently covered in an EPA-sponsored National Symposium on Wastewater Aerosols and Disease in Cincinnati, Ohio, September 19-21, 1979. There have been several programs directed at the measurement of aerosols from activated sludge treatment plants and spray application of wastewater to land. These studies have included aerosol monitoring efforts and attempts to examine health effects in populations either working at the site or living nearby. In the case of spray application of wastewater, these efforts have led to the conclusion that if untreated wastewater is aerosolized, measurable levels of pathogenic viruses will be produced and some of the pathogens will be transported downwind. Health studies have not produced conclusive results, but some have shown the possibility of adverse health effects, particularly the studies in Israel. These studies have had deficiencies in their designs, primarily

in that they lacked sufficient sensitivity to measure the appropriate health indicators. A study has recently been initiated to examine aerosols from land application of treated municipal wastewater both from monitoring and health effects points of view. This program will be conducted as part of a Lubbock Christian College Institute of Water Research effort to study the City of Lubbock, Texas, domestic effluent wastewater land treatment system. This four-plus year effort should provide many answers relative to the health risks of people living near aerosolized wastewater and additional data on types, quantities, and distances transported for various pathogens.

Considerably less effort has been performed to study the spray and tank truck application of liquid municipal sludge to land. An aerosol monitoring effort has been performed at a site near Chicago by the Metropolitan District of Chicago, but the results are not yet available. No information was available at the beginning of this program for the possible formation of aerosols via the application of liquid sludge by tank trucks. This program was directed toward examining possible aerosol formation during spray application of liquid sludge by tank trucks and by spray guns. Information from this effort could be utilized to assist in the assessment of possible health problems to personnel applying wastes and to populations living near disposal sites from this manner of disposal. This assessment would assist the EPA in the formulation of guidelines for the safe disposal of municipal sludge.

RATIONALE FOR THE STUDY

This study was initiated to provide information on the generation of harmful microbial aerosols near sites practicing land application of liquid municipal sludge. Very little information is available regarding the production of microbial aerosols as to the types or quantities of organisms produced. Because of the importance of land application of municipal sludge, the EPA must collect the necessary data to answer the safety questions. If problems are identified, then it is likely that changes in the mechanism in applying sludge can be made using current technology.

STUDY OBJECTIVES

The original objective of this study was to determine if the use of tank trucks in applying liquid sludge to land resulted in the formation of pathogenic microbial aerosols. A secondary objective was to characterize the sludge from several sites practicing land application for the presence and levels of microbiological constituents, trace metals, and pesticides.

During preliminary site evaluation, sites were identified which utilized high-volume spray guns to apply liquid sludge. The program objectives were expanded to include evaluation of aerosols generated at spray application sites and to compare these results to those from the truck sites and to sites conducting spray application of wastewater.

STUDY DESIGN

The study was conducted in four phases: 1) development of microbiological analysis methods; 2) survey of sites practicing land application of liquid sludge; 3) field sampling of selected sites; and 4) data analysis and interpretation.

The initial work required that analytical methods be developed or modified for the analysis of sludge for bacteria and viruses. This developmental work was conducted on representative sludge samples and was directed at preparing a uniformly dispersed sludge sample which would allow optimal quantitation of the bacteria and selection of appropriate media for analyses.

Site survey was conducted in a preliminary manner by telephone contact with EPA, state, and local offices. Key people at the potential sites were then contacted to determine if they would be willing to participate in the study and if their site would be suitable with regard to sampling considerations and levels of organisms expected in the sludge. The original design called for six tank truck sites to be selected for evaluation and three to be selected from these for aerosol monitoring. It was subsequently learned that there were several sites practicing spray-gun application of sludge, and the original design was modified to include four sites for monitoring, two tank truck sites and two spray gun sites.

Based on the results of telephone inquiries, six sites were visited and evaluated for their suitability. A sludge sample was obtained at each site for the characterization study and arrangements were made for a second sample to be collected by plant personnel at a later time.

Final selection of the four sites for aerosol monitoring was made based upon the information obtained during the site visit and on the levels of microbiological components of the sludge that could be expected based on the results of the preliminary analyses.

Aerosol monitoring was to be accomplished by utilizing high-volume electrostatic precipitator-type samplers. Seven microbiological aerosol sampling runs were scheduled at each site and a special run for collection of viruses at one spray gun site. At each site, samplers were set up to monitor background levels and downwind levels of the aerosols, with the downwind samplers located as close as possible to the sprayed area at the tank truck sites and at 50 m and 100 m downwind of the spray edge at the spray gun sites. The collecting media from two to three samplers were pooled to increase the sensitivity of the analyses. At the last two sites a randomization scheme was used to allocate samplers to sampling stations so that effects of sampler bias would be minimized. All samplers were run at the same location for the enterovirus run, and all collection media were pooled for analysis. High-volume particulate samplers were used at the spray gun sites to give an estimate of the levels of particulate matter present at the sampling locations.

The results were submitted to statistical analysis to evaluate whether microbiological aerosols were being generated at the sites, compare the two types of sites for levels of microbiological aerosols, if appropriate, and compare the results to those from sites practicing spray application of wastewater.

PARTICIPATING ORGANIZATIONS AND PRINCIPAL PERSONNEL

The research effort described in this report has been performed by Southwest Research Institute with significant support and contributions from several other organizations. The following is a list of these participating organizations:

Southwest Research Institute (SwRI), San Antonio, Texas
Department of Environmental Sciences
The University of Texas at San Antonio (UTSA), San Antonio, Texas
Center for Applied Research and Technology (CART)
Naval Biosciences Laboratory (NBL), Oakland, California
Cibolo Creek Municipal Authority, Cibolo, Texas
O. J. Riedel Wastewater Treatment Plant
City of Portland, Oregon
Bureau of Wastewater Treatment
Longmont Wastewater Treatment Plant, Longmont, Colorado
Department of Public Utilities, Shreveport, Louisiana
Muncie Sanitary District, Muncie, Indiana
Public Utility Department, City of St. Petersburg, Florida

The principal participating personnel and their areas of responsibility are shown below:

SwRI Department of Environmental Sciences

D. E. Johnson, Ph.D. - Principal Investigator
H. J. Harding - Director of Field Activities
R. E. Thomas - Statistician

UTSA - Center for Applied Research and Technology

C. A. Sorber, Ph.D. - Director of Analysis
B. E. Moore - Sample Analysis

NBL

M. A. Chatigny - Consultant in Aerobiology and loan of aerosol samplers

SECTION 2

CONCLUSIONS

SLUDGE CHARACTERIZATION STUDY

All six sites and all samples contained measurable levels of cadmium, copper, mercury, nickel, lead, and zinc. The mean concentrations ranged from 15.7 µg/g for mercury to 9,180 µg/g for zinc, on a dry weight basis. Molybdenum was detected in only three samples, at concentrations of 40, 48, and 140 µg/g, dry weight.

Of the 16 priority pollutant pesticides and seven polychlorinated biphenyl formulations screened for in the sludge, only chlordane and Aroclor 1248 were detected in any of the samples. The chlordane concentration in sludge fell between 11 and 16 µg/g in the four samples in which it was detected. Both samples from one site (Muncie, Indiana) were found to contain AR 1248, at 25 and 26 µg/g.

Microbiological screens of sludge samples from all sites contained four non-enteric bacteria and eight enterobacteria organisms. Other organisms were found in samples from one or more of the sites.

AEROSOL MONITORING

Generation of microbiological aerosols most likely does occur at sites utilizing tank trucks, but results in very low bacterial aerosol levels. Monitoring at these sites is difficult due to the fact that a truckload of sludge is applied over a short period of time while the truck passes over several hundred meters of land.

There was strong evidence for aerosolization of microbiological organisms at the spray application sites, notably among the fecal coliform, fecal streptococci, and mycobacteria results. Fecal coliform and fecal streptococci were detected downwind at both sites, while at Portland they were not consistently detected at the background site and at St. Petersburg were approximately two orders of magnitude lower at the background sites. Mycobacteria data at St. Petersburg gave similar results to the fecal coliform data.

On a special virus run, 1,470 m^3 of air was sampled and no human enteric viruses were detected from the pooled sample. This converts to a concentration of less than 0.0016 pfu/m^3 of air, and the implication is that aerosolization of viruses does not present a significant problem.

MODELING OF AEROSOL DATA

Modeling of sludge aerosols presented problems at both types of sites. At the tank truck sites, there was no indicator of aerosolization efficiency and only relative impact factors could be calculated. At the spray sites, particulate data from high-volume samplers were used to model the results, but this required several assumptions which led to uncertainty in the results.

The impact with respect to standard plate count of tank truck application of sludge was considerably lower than that of spray application of wastewater. The reasons for this could relate to the matrix, the means of application, or a combination of these or other factors.

Mean aerosolization efficiencies at the spray site were estimated to be 0.00070 percent and 0.037 percent at the Portland and St. Petersburg sites, respectively, based upon the particulate data. These are lower than at comparable sites applying wastewater, but the method of calculation and the interpretation do not allow strict comparability of the results.

SECTION 3

RECOMMENDATIONS

Where tank truck application is exercised as the means of sludge disposal, the generation of aerosols is likely to be minimal. If further reduction is deemed necessary, the option of subsurface injection should be considered.

Where spray application is the means of disposal certain practices may be observed to reduce formation of and exposure to aerosols, e.g., creation of a buffer zone around the application site, application as much as possible under ideal meteorological conditions, and the use of low-pressure, downward-directed nozzles.

The presence of microbiological organisms in the sludge, as well as toxic metals, pesticides, and polychlorinated biphenyls (PCB's), suggests that the composition of the sludge should be well known before land application is accomplished. In addition to aerosol generation, there is a potential for harmful effects through soil and ground water, and there should be some assurance prior to land application that levels of these contaminants do not exceed tolerable levels.

SECTION 4

STUDY SITES

SELECTION PROCESS FOR STUDY SITES

A multifaceted approach was used to locate sites suitable for inclusion in the study that would meet the scope of work as originally proposed and subsequently expanded to include two types of application methods.

The first phase of the approach utilized a computerized literature search. As a result of this search, numerous articles were obtained which furnished potential study sites, names of USEPA and state government officials knowledgeable in sludge disposal, and names of academic personnel associated with research in sludge recycling. Once potential study sites were listed, phone contacts were then made with appropriate personnel to determine suitability of the site for inclusion in the study.

Based on consent to being a participating site in the study and a firm commitment to land application for the duration of the study, six sites were chosen for sludge characterization and evaluation for future aerosol monitoring. Four of these six sites were then chosen for aerosol monitoring based on evaluation of potential aerosol formation factors. A description of each of the six original study sites follows.

SITE DESCRIPTIONS

Tank Truck Sites

Longmont, Colorado--

The City of Longmont, Colorado, located approximately 80 km (50 miles) north of Denver, operates a single stage trickling filter wastewater treatment plant with an average flow of 30,000 to 34,000 cubic meters per day [8 to 9 million gallons per day (MGD)]. This plant treats all of the wastewaters generated in the Longmont area which are 85 to 90% domestic in origin. The 10 to 15% balance is industrial in nature discharged mainly by fast food restaurants and food processing plants.

The first step in the treatment process is flow measurement in a Parshall flume followed by bar screens to remove large objects. After sand and fine settleable particles are removed in grit removal chambers, compressed air is used for mixing and oxygen uptake of the

wastewater. After the suspended matter is reduced in size by a comminutor, the wastewater is pumped to a flow equalization tank where recirculation prevents solids from settling. The flow through the plant is constant from this point on.

After leaving the flow equalization tank, alum and an organic polymer are added to enhance settling of suspended matter in two parallel primary clarifiers. Clarifier effluents are combined and then divided (60:40) between two trickling filters with redwood slats as media. Trickling filter effluents are combined and flow through basins with Bio-Discs, three pairs in series with each of the pairs rotating in opposite directions. Prior to final clarification, alum and polymer are added again and after disinfection of final clarifier effluent in a chlorine contact chamber, the treated wastewater is discharged into the St. Vrain River. Sludge from final clarification and sluff from the Bio-Discs are returned to the plant headworks.

Only settleable solids from the primary clarifier are digested. Treatment prior to digestion includes grinding and thickening, followed by primary digestion at 35 to 36°C (95-97°F) with recirculation for 15 days. Another 15 days at 34°C concludes secondary digestion. Approximately 38 to 57 cubic meters (10,000 to 15,000 gallons) of digested sludge are available daily for land disposal.

Longmont utilizes two Big Wheels®, each with a capacity of 5.7 cubic meters (1,500 gallons), for land application in the spray mode only. Sludge is applied to private land whenever feasible, with the condition that the landowner pays for fuel used to transport and spread the sludge. Typically, two types of farm land receive sludge as a fertilizer and soil conditioner: fodder-producing farm land and sod farms. At the time of the aerosol study at this site, sludge was applied on a 240 hectare (600 acre) plot in the Rocky Mountain foothills producing wheat for use as cattle fodder. The addition of a 6,000-gallon tanker is planned to alleviate wear and tear on the Big Wheels® caused by the distance of the sludge application sites from the treatment facility.

Muncie, Indiana--

The Muncie Sanitary District has the responsibility for treatment and disposal of most wastewater generated in the Muncie area, an industrial area in central Indiana 100 km (60 miles) northeast of Indianapolis. To perform this responsibility, the District operates an activated sludge treatment plant with an average daily flow of 60,000 cubic meters per day (16 MGD). It is estimated that 40% of the influent stream is industrial in origin. However, due to strict enforcement of an industrial waste ordinance, the load of toxic materials on the activated sludge process is kept minimal resulting in less operating problems with the plant.

On a daily basis 227 cubic meters (60,000 gallons) of digested sludge are ready for disposal and when conditions permit, the entire

amount is land applied. Due to odor problems associated with surface application and a prevailing wind direction of south (S) to southwest (SW), the District attempts to apply sludge on sites northeast (NE) of town even though this requires that the sludge be hauled through town. During 1979 the District was leasing a 46-hectare (113-acre) site 16 km (10 miles) northeast of the city as a land application site. This plot of tight clay soil supported no crop production during the application period.

Two types of trucks are used for land application: 23-cubic meter (6,000-gallon) tankers with detachable tractors and 6-cubic meter (1,600-gallon) tanks permanently mounted on flat bed trucks. To discharge the sludge from a slowly moving tanker, it is allowed to flow by gravity through a 15 to 30 cm (6 in. to 12 in.) diameter short pipe controlled by a valve. A fan-shaped nozzle is attached to the end of the pipe to create a wider dispersion path of the sludge stream.

At the site the driver would stop at one end of the application plot, manually open a valve at the rear of the tanker, and then proceed to drive in a straight path across the field. In traveling at a speed of 5 to 7 km/hr (3 to 4 mph) during a sludge application traverse, the 23-cubic meter (6,000-gallon) tankers applied on an area 2 to 3 m (7 to 10 feet) wide and 600 m (670 yards) long. Actual application time averaged about 6 minutes. The smaller trucks would empty their loads in approximately 1.75 minutes while traversing a distance of 200 m (220 yards).

The availability of equipment and personnel determined the number of loads hauled and the amount of sludge applied. On a typical day two of the large tankers and two small trucks land applied. Even though a 32-km (20-mile) round trip was required for each load, the arrival of the trucks at the site was not sufficiently staggered to eliminate concurrent application by two or more trucks. In fact, at times land application would be performed by one large tanker and two small trucks at the same time. As conditions permit, the applied area is disked, usually two to three days after application. When climatic conditions permit, application occurs during the winter months, though disking cannot be performed due to frozen ground.

Cibolo, Texas--

The Cibolo Creek Municipal Authority (CCMA) operates a two-stage trickling filter process wastewater treatment plant located about 24 km (15 miles) northeast of San Antonio, Texas. Due to the suburban character of the district, most of the influent is domestic in nature though all wastewater generated on Randolph Air Force Base is treated by this facility. The average flow is 7,200 cubic meters per day (1.9 MGD) with a 8,100 cubic meters per day (2.15 MGD) design capacity. Recently completed construction of a smaller adjacent plant in September 1979, resulted in an additional capacity of 3,800 cubic meters per day (1.0 MGD).

The first part of the treatment scheme is removal of large particles by a bar screen in the headworks. Next, grit and sand are removed in a grit chamber followed by primary clarification and flow through the primary trickling filter containing a coarse PVC media. Effluent from the primary trickling filter flows through a secondary trickling filter containing fine PVC media with twice the surface area of the media in the primary trickling filter. Final clarification is followed by disinfection in a chlorine contact chamber, flow measurement in a Parshall Flume, and final discharge into Cibolo Creek.

Settleable solids from only the primary clarifiers are transferred to one of two anaerobic digesters on a timed cycle basis. An unmixed and unheated digestion period of 20 to 30 days occurs with a weekly sludge production of 38 cubic meters (10,000 gallons). The supernatant from the digesters and sludges from secondary and final clarifiers are combined and returned to the headworks of the plant.

Two or three times weekly approximately 15 to 19 cubic meters (4,000 to 5,000 gallons) of digested, stabilized sludge are land applied on a 1.6-hectare (4-acre) portion of a 12-hectare (30-acre) tract adjacent to the plant. This land is dedicated solely to sludge disposal and can be cropped for hay production when desired. A Big Wheels® truck with a capacity of 5.7 cubic meters is used for application utilizing either the spray attachment or subsurface injectors depending on weather and field condition. The sludge contained 3 to 5% solids of which 40 to 60% is volatile.

Spray Irrigation Sites

Portland, Oregon--

The Bureau of Wastewater Treatment is responsible for the operation of the Columbia Boulevard Treatment Plant, an activated sludge process plant designed to give secondary treatment to 378,500 cubic meters of wastewater (100 MGD) per day, with a hydraulic capacity of 1,136,000 cubic meters per day (300 MGD) through the primary section, and 757,000 cubic meters per day (200 MGD) through the secondary section.

The first step in treatment of domestic and industrial wastewater from the Portland area is to remove large objects using grit removal chambers followed by four mechanical screens. Next, primary clarifiers separate heavy solids that settle as well as grease and oil that float. The settleable material, or sludge, is pumped to thickeners for further concentration following fine grit removal. The wastewater is then mixed with activated sludge in aeration tanks. From the aeration tanks, the mixed liquor sewage flows to final clarifiers where microorganisms and solids settle to the bottom. The final clarifier effluent is chlorinated and then discharged into the Columbia River.

Raw sludge from primary clarification is degritted, gravity thickened, preheated to 35°C (95°F) and anaerobically digested at 38°C ± 0.5 (100°F ± 1°) with gas mixing. After approximately 18 days of primary digestion,

the sludge is transferred to secondary digesters for an average detention time of 36 days with no external heat. Anaerobic digester supernatant is returned to the treatment process ahead of the primary clarifiers to provide seeding and buffering material to the raw sludge collected in the clarifiers. Waste-activated sludge is thickened by centrifuges, anaerobically digested, and then transferred to a sludge storage lagoon. This sludge is not currently being land applied.

During the period of this study, digested sludge from the anaerobic digesters was hauled by tank truck to Port of Portland property adjacent to Terminal 6 and spray irrigated on dredging spoils for sand stabilization. On a typical day 14 to 16 loads of 22.7 cubic meters (6,000 gallons) each were land applied when trucks and personnel were available. A portable diesel-powered pump with a rated capacity of 1.1 cubic meters per minute (300 GPM) was used to pump the sludge from the truck to a single spray gun with a 3.2 cm (1.25 in.) diameter nozzle. The time of spraying was dependent on the length and the size of the transfer line between the pump and the nozzle but varied between 10 and 15 minutes. Typically, the nozzle would generate a spray radius of 61 meters (200 feet) while rotating 4 to 5 RPM. The spray gun was moved daily and the applied area was disked the following day to promote evaporation of any standing water and alleviate odor problems.

Shreveport, Louisiana--

A new treatment facility, the Lucas Wastewater Treatment Plant, is operated by the Department of Public Utilities, City of Shreveport. This plant has a design capacity of 91,100 cubic meters per day (24 MGD) and is currently hydraulically loaded at a 30,000 cubic meters per day (8 MGD) rate. The majority of influent is domestic in origin, since most local industry treats and disposes of their own wastewaters. However, some increased biochemical oxygen demand loading is occasionally experienced due to a discharge from a creamery. This plant is currently underrated hydraulically and treats only about 30% of the wastewater generated in Shreveport. Improvements are being made to the collection system, and a series of lift stations are currently being installed. As these lift stations are completed, the hydraulic load of the plant will rapidly approach the design capacity.

The plant is the activated sludge type without primary clarification resulting in complete mix. The treatment scheme consists of: a Parshall flume for flow measurement, aerated grit basins to remove sand and other heavy inorganic material from the raw wastewater, comminutors to reduce the size of solids, and four aeration basins with surface mixing and mechanical aeration. These processes are followed by final clarification basins, chlorine contact chambers, and discharge into the Red River.

An aerobic, two-stage digestion facility handles all the solids from the treatment facility. Prior to digestion all raw sewage solids are aerated in the aeration basins. Between 70 and 90% of the waste-activated sludge is returned to the aeration basin while the remaining

10 to 30% is transferred to the digester. Aerobic digestion of the waste activated sludge and scum from the scum well takes place in one of two two-stage basins. Four 370-kw (500-hp) squirrel cage blowers, each rated at 230 cubic meters per minute, are utilized for aeration of the sludge. Current detention time is estimated at 11 days (November 1978) but was to have been increased to 21 days. Daily volume of digested sludge to be wasted is about 380 cubic meters (100,000 gallons) of about 2.5-percent solids content. Transfer of sludge from the digester to the storage lagoon is on an unscheduled basis.

The sludge application site is 160 hectares (400 acres) in size and is located 13 km (8 miles) from the Lucas Plant. The soil on the site consists primarily of Norwood silts and Moreland clay. A storage lagoon with a capacity of 7,600 cubic meters (2×10^6 gallons) and maximum liquid detention time of 60 days is centrally located on the site.

Two systems are utilized for land application of the lagooned sludge: subsurface injection and a spray system. The injection system consists of a seven sweep unit rated at 3 cubic meters per minute (800 GPM) mounted on a modified crawler tractor which tows a heavy-duty flexible hose. The alternative spray system consists of a rotating nozzle which uniformly distributes the sludge over a circular area 144 m (472 ft) in diameter at 3 cubic meters per minute. Nine nozzles provide the site cover and automatic timing devices allow a switch of flows from one nozzle to another to achieve a uniform loading rate. Spray nozzles mounted on trailers will be used to apply sludge to areas where the fixed nozzles fail to cover.

Originally, the primary application system was to have been subsurface injection with the spray irrigation system utilized as a back-up system. However, personnel operating the system anticipate reversing system priorities and using the spray system as the primary method for land application. The primary reason for this role reversal is that spray irrigation would not cause turf damage as would subsurface injection, and secondarily, but very critical, is that fewer mechanical problems are anticipated with the spray irrigation equipment.

St. Petersburg, Florida--

The Department of Public Utilities, City of St. Petersburg, operates a 24-hectare (60-acre) land application site for disposal of digested sludge from three sewage treatment facilities in the St. Petersburg area. All three plants are of the activated sludge type and anaerobically digest sludge from primary clarification.

Digested sludge from these STP's is trucked to a landfill site near the spray field where it is stored in a lagoon of unknown capacity until land applied. To reduce stagnation and layering of the sludge, a recirculating pump is operated at the lagoon. On a daily basis during the week, sludge is applied to a portion of the 24-hectare site through

two of the available spray guns, forty of which are fixed and two are portable. Lagooned sludge is then sprayed for a four- to six-hour period at a nominal spraying rate of 1.9 cubic meters per minute (500 GPM) for each spray gun. The radius of the spray pattern would vary between 26 m (85 ft) and 45 m (150 ft) with 36 m (120 ft) as the average.

Various ground covers are used at this site for nutrient and water uptake to help alleviate potential ground water contamination. In cooperation with the USDA Soil Conservation Service, different types of grasses are being grown in experimental sections of the site as potential fodder or compost material.

SELECTION PROCESS FOR AEROSOL MONITORING SITES

After the initial site visits and completion of microbiological assays of both sets of sludge characterization samples, two sites of each type of application process were chosen for aerosol monitoring. Factors influencing this decision included: 1) total time of aerosol generation each day, 2) commitment to land application for the duration of the study, 3) relative ease in sampling aerosols at the study site, 4) experience of personnel involved in the land application process, and 5) level of microorganisms, a secondary influence since relative concentration differences were minor.

As a result, Portland and St. Petersburg were selected as the spray gun sites and Cibolo and Longmont as the truck sites to be monitored.

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SECTION 5

METHODS AND MATERIALS

SAMPLE COLLECTION AND HANDLING METHODS

Sludge Characterization Samples

To assist in determining which four of the six initial study sites were to be monitored in the follow-up aerosol study, it was necessary to determine levels of microbial pathogens and viruses from liquid sludge samples collected at each site.

This task was accomplished by collecting two sets of grab samples approximately six weeks apart during the second phase of this study. The first samples were collected during the initial site visit at which time arrangements were made for subsequent collection of a second sludge sample. Additionally, each site was evaluated for its suitability for inclusion in the aerosol monitoring task.

Each set of liquid sludge samples consisted of four one-liter aliquots in sterile polypropylene containers for microbiological analyses, one one-liter aliquot in an acid-washed polypropylene container for trace metals determination, and a solvent-rinsed glass bottle with a Telfon-lined cap for pesticide and PCB analysis. Following collection, these samples were cooled to 4°C in an ice-water bath, packed in an insulated container with one frozen Kool Pac®, and shipped to San Antonio via counter-to-counter airline parcel service with samples arriving at the UTSA-CART laboratories early the following day. This allowed for no more than 24 hours between sample collection and start of microbiological assays.

At the Muncie, Longmont, Cibolo, and Portland sites, sludge samples were collected as a tanker truck was being loaded. At the Shreveport and St. Petersburg sites sludge samples were collected from the irrigation system between the storage lagoon and the spray irrigation nozzles or at the nozzle head.

Sludge Samples During Aerosol Monitoring

During each aerosol run, sludge samples were collected from some part of the system supplying sludge to the aerosol source. This was necessary to determine the source strength concentrations of those microorganisms being assayed in the aerosol samples.

At the Portland, Longmont, and Cibolo study sites, one liter of sludge was collected for each tank truck applied at the study site. Since land application at the St. Petersburg site occurred continuously, grab samples were obtained from the irrigation system at two times during the aerosol run. At the end of a run, the one-liter sludge aliquots were composited. Two liters of the composite were placed in sterile one-liter containers and cooled to 4°C prior to shipment with the aerosol samples.

High-Volume Aerosol Samplers

One type of high-volume air sampler, the Litton Model M large-volume air sampler, was used for collecting biological aerosols in this monitoring program. These were manufactured by Applied Science Division, Litton Systems, Inc., Minneapolis, Minnesota. For purposes of this study, 10 Model M samplers were loaned to SwRI by the Naval Biosciences Laboratory, Naval Supply Center, Oakland, California.

The sampler is designed to collect airborne particles and concentrate them into a thin, moving film of liquid media. The collection and transfer medium selected was brain-heart infusion (BHI) broth with 0.1-percent Tween 80®, which was shown to be adequate for sample concentration and for preservation and assay of the microorganisms (3). The nominal air flow rate for the samplers is 1,000 liters per minute (lpm) (=1.0 cubic meter per minute), and during the typical aerosol run of 30 minutes, a total air volume of 30,000 liters (=30 m^3) was sampled. A complete description of the sampler is provided in Appendix A.

Collection Efficiency of Aerosol (Model M) Samplers

All aerosol results were corrected for the collection efficiency of the Model M large-volume air samplers relative to the all-glass impinger (AGI), a common aerosol collector. The Model M was determined to be less than 100% efficient in collection of viable microbes contained in or on particles. To determine the collection efficiency, Model M samplers were operated simultaneously with AGI's in the controlled conditions of a wind tunnel at the Naval Biosciences Laboratory, Oakland, California, using Flavobacterium sp. as a test organism. Based on a series of runs conducted in the summer of 1976 (EPA Interagency Agreement IAG-D7-0701, "Evaluation of Health Effects Associated with the Application of Wastewater to Land") and repeated in the summer of 1980 (EPA Grant S806204-010, "Health Effects Study for the Lubbock Land Treatment Project"), an average collection efficiency of 45% was determined for the Model M samplers. All aerosol data tabulated and presented in the Results section have been adjusted by this factor to account for the collection efficiency.

Field operation of the samplers first required that an effective decontamination be performed followed by suitable storage in this sterile state. This was accomplished by a cleanup procedure using both absolute

ethanol and a buffered sodium hypochlorite solution, followed by sealing all sampler openings. Samplers were normally decontaminated at the staging area of each study site at the conclusion of an aerosol run. When more than one run was conducted on the same day, decontamination was performed in the field between runs. A copy of the step-by-step cleanup procedure can be found in Appendix B.

After sampling positions were assigned according to site type, Model M samplers were placed in the center of a level portable field table. With this arrangement, the air inlet was 1.5 meters (5 feet) above ground level. To supply 115V power for sampler operation, a portable, gasoline-powered alternator was located approximately 5 meters downwind of each sampler location.

The Model M samplers were deployed in the sampling array and operated simultaneously for about 30 minutes to constitute an aerosol run. Sampling was always conducted at an air flow rate of 1.0 m^3/min and an electrostatic potential of 12 to 14 kilovolts. A more detailed description of the procedures for sampler setup and operation can be found in Appendix C.

Meterological Measurements and Instrumentation

Simultaneous meteorological measurements were made and recorded continuously at two downwind locations on a two-meter tripod during each aerosol run. At sludge application sites utilizing fixed sprayers where the downwind sampling array was at 50 meters and 100 meters, the two-meter meteorological stations were located at 50 meters between the right and left sampling locations and near the 100 meter station. Where tank trucks were used for sludge application, the meteorological stations were placed adjacent to the two downwind sampler locations. The following parameters were measured on each station: wind speed (derived from wind run versus time plot), wind direction, and temperature. Atmospheric stability can be inferred from the variation in wind direction. Wind velocities assist in determining the probable downwind and crosswind axes of the aerosol plume.

Measurements were made and recorded on time-referenced strip charts contained in mechanical weather stations, Model 1071, manufactured by Meteorology Research, Inc. (MRI), Altadena, California. These weather stations were held on rigid tripods at two meters above grade. This arrangement permitted easy access to the instrumentation for alignment, servicing and, chart changing.

Starting threshold for both the anemometer and wind vane is less than three-fourths of a mile per hour. Wind direction measurement accuracy is ±1 percent of full scale while wind speed data are accurate to ±2 percent.

Wind direction and wind speed were determined from the MRI strip charts on an average basis for each period comprising the aerosol run.

These periods varied in length from three minutes at the tank truck sites to 15 minutes at the continuously spraying spray gun sites. A wind direction range was also determined for each sampling period.

Field measurements of solar radiation were recorded by a Belfort Pyrheliograph Model 5-3850 with a range of 0 to 3 gram-calories per square centimeter per minute. This instrument was located in an area free from solar obstructions at a downwind station during each aerosol run. An average solar radiation value was determined for each period of the aerosol runs. The values for the periods within each run were then averaged and recorded to represent a solar radiation figure for each aerosol run.

A Bendix Model 566-2 portable, electrically-aspirated psychrometer was used to observe wet and dry bulb temperatures downwind of the sludge aerosol source. These readings were recorded for each period of aerosol sampling during segmented runs and every 15 minutes when continuous aerosol formation was being sampled. The reported measurements were the dry bulb temperature and the percent relative humidity (as determined by comparison of the wet and dry bulb temperatures).

During each run, visual observations were made of cloud cover, cloud type, and minimum cloud height based on the cloud type. Cloud cover was estimated as the number of eighths of the whole sky covered by any type of cloud formation. Cloud types and cloud heights were determined by visual observation and comparison with photographs found in the Manual of Cloud Forms and Codes for States of the Sky (4).

ANALYTICAL METHODS

Sludge Characterization Samples

Bacteriology--

Sample Processing--Before beginning work with individual bacterial tests, a method was sought which would allow preparation of a uniformly dispersed sludge sample. The procedures examined included vortex mixing with sterile 3-mm glass beads, homogenization in a Waring blender, and sonication. A variety of sludges and standard bacterial tests were used. In all cases, an increase in the number of bacterial colonies observed in a processed sample over the number observed by direct plating of the unprocessed sample was interpreted as an improved technique.

After initial testing, sonication was excluded from further experimentation. Results presented in Table 1 show that even at the lowest frequency tested, a significant loss of both fecal coliform and total plate count was observed.

TABLE 1. EFFECT OF SONICATION ON BACTERIAL VIABILITY

Power Output (% of maximum*)	Length of Sonication (minutes)	Fraction Surviving (% of direct plating) Fecal Coliform	Total Plate Count
34	1	39	11
	5	27	8.1
	10	13	0.7
39	1	42	12
	5	4.7	6.2
	10	1.4	2.8
45	1	31	10
	5	3.7	3.2
	10	0.5	2.1

* Branson Sonifier Model 2000, maximum output 150 watts.

Representative results obtained in handling sludge samples by vortex mixing and blender homogenization are presented in Table 2. Overall, vortex mixing with glass beads was the best method for obtaining a uniform sample prior to bacteriological examination. Of the total number of tests run, 75% showed higher recovery of viable bacterial cells by vortex mixing.

Based upon these results, a standard procedure was developed. A volume of all sludge samples (10-25 mL) was placed in a sterile tube containing glass beads and vortex mixed at high speed for 2 minutes. Bacterial assays were completed with the resultant sample.

Indicator Organisms--Total coliforms, fecal coliforms, and fecal streptococci were recovered from vortexed sludge samples using three different methodologies: multiple-tube fermentation [Standard Methods, 14th edition (5)], membrane filtration (Standard Methods, 14th edition), and direct spread plating onto appropriate selective media.

Media used for these tests were those specified in Standard Methods, 14th edition, except for the selection of m-Enterococcus agar as a plating medium for the enumeration of fecal streptococci. Recent comparisons of this medium with KF agar demonstrated its superior recovery of fecal streptococci. Additionally, tubes from the confirmed multiple-tube fermentation procedure were subjected to a completed test by transferring a loopful of medium from each type of the highest positive dilutions onto appropriate selective media. Growth of typical indicator colonies allowed calculation of a "completed" most probable number (MPN) value.

Comparative results from representative trials are presented in Table 3. In the majority of tests, values for completed MPN tests

TABLE 2. COMPARISON OF SLUDGE PROCESSING TECHNIQUES

Sample-- Method of Treatment	Fraction Surviving (% of direct plating) Total Plate Count	Fecal Coliforms	Fecal Streptococci	Enteric Bacteria*
Digested Sludge--				
Vortex mixing†	242	154	87	--
Blender homogenization (3 min)	158	100	103	--
Wasted Sludge--				
Vortex mixing	163	200	150	151
Blender homogenization (10 sec)	66	109	85	100
Blender homogenization (3 min)	146	91	120	119

* Plate count on Eosin Methylene Blue (EMB) agar, incubated at 35°C.
† Processed at high speed for 2 minutes.

TABLE 3. ENUMERATION OF INDICATOR ORGANISMS - METHODOLOGY COMPARISON

Indicator Organism	Multiple-tube Fermentation (MPN/100 mL) CONFIRMED	Multiple-tube Fermentation (MPN/100 mL) COMPLETED	Membrane Filtration (cfu/100 mL)	Direct Plating (cfu/100 mL)
Total coliforms				
Run 1	7.9×10^7	1.3×10^8	1.8×10^8	2.9×10^8
Run 2	2.4×10^8	3.5×10^8	1.5×10^8	3.5×10^8
Run 3	1.3×10^8	2.4×10^8	2.6×10^8	4.2×10^8
Fecal coliforms				
Run 1	5.4×10^7	9.2×10^7	4.6×10^7	5.0×10^7
Run 2	1.3×10^8	2.4×10^8	3.8×10^7	4.2×10^7
Run 3	3.4×10^7	1.3×10^8	5.3×10^7	7.7×10^7
Fecal streptococci				
Run 1	9.4×10^4	1.4×10^5	4.5×10^6	4.3×10^6
Run 2	1.4×10^7	1.4×10^7	3.0×10^6	3.0×10^6
Run 3	2.8×10^5	2.8×10^5	5.2×10^6	8.8×10^6

were higher than those obtained using the standard confirmed MPN procedure. With the exception of fecal coliforms, a direct spread plating procedure resulted in recoveries equal to or greater than the other methodologies tested. Based on past experience, the assay of fecal coliform by direct plating could be improved by preincubating inoculated plates at 35°C for 4-5 hours prior to incubation at 44.5°C.

A significant advantage of direct plating over multiple-tube fermentation procedures involved handling time. With direct plating, typical indicator colonies could be counted directly off plates following incubation. On the other hand, MPN procedures involved transferring samples from presumptive to confirmatory media over several days incubation time. Optimal results required additional plating from confirmed tubes to obtain a completed test.

Based on these considerations, direct plating on appropriate selective media was used for enumeration of indicator organisms in sludge. Samples were diluted in sterile phosphate-buffered saline (pH 7.3 ± 0.1) and 0.1-mL aliquots were spread over each of the three replicate plates. Total coliforms were assayed on m-Endo agar LES, fecal coliforms on m-FC agar, and fecal streptococci on m-Enterococcus agar. Incubation was at 35°C for 24 hours for total coliforms. Fecal streptococci were counted after incubation for 48 hours at 35°C. Fecal coliform plates were incubated at 35°C for 4-5 hours and then transferred to 44.5°C for an additional 20 hours of incubation.

Standard Plate Count--Appropriate serial dilutions of the processed sludge sample were prepared in phosphate-buffered saline and plated in triplicate. Inoculation into plate count agar was conducted in accordance with Standard Methods, 14th edition. Incubation was at a temperature of 35°C ± 0.5°C for 48 hours. Plates were placed in the incubator without crowding.

Only plates showing 30 to 300 colonies were considered in determining the standard plate count. The results as reported were the average of all plates falling within these limits. Results were reported as colony-forming units (cfu) per gram of total suspended solids.

Salmonella-Shigella--Since concentration of sludge samples by filtration through diatomaceous earth was not feasible, the sensitivity of proposed procedures for Salmonella and Shigella species was reduced. Enrichment procedures schematically depicted in Figure 1 were modified to allow quantitation (by range) of these organisms. Processed sludge samples were inoculated directly into two enrichment media: selenite broth and GN broth. Volumes of 25 mL, 10 mL, 1 mL, and 0.1 mL or 10 mL, 1 mL, 0.1 mL and 0.01 mL were inoculated as appropriate. Enrichment media were held at 37°C for approximately 18 hours.

After enrichment, an aliquot from each bottle was dilution plated onto appropriate media for selection of isolated colonies. Following incubation at selective temperatures, colonies were subcultured and

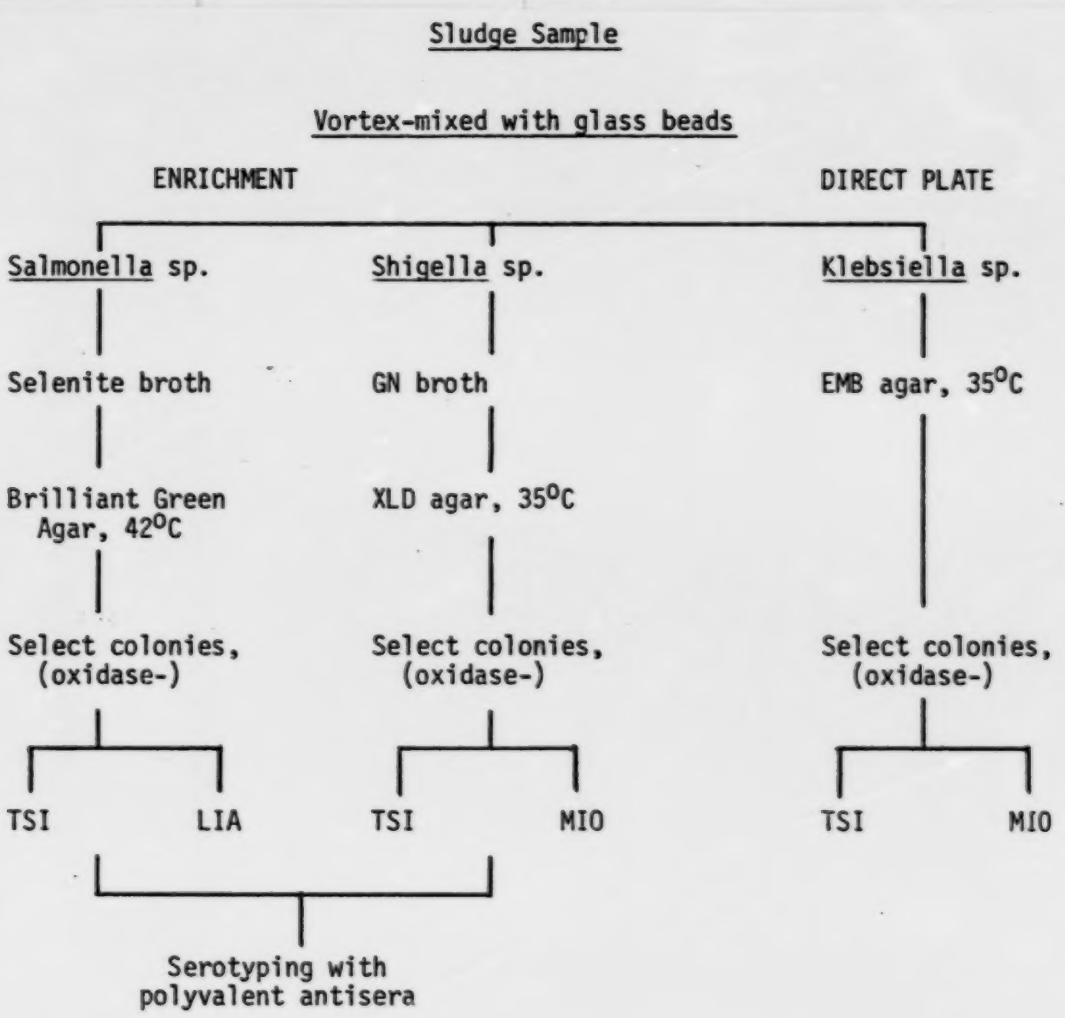

Figure 1. Schematic for isolation of Salmonella, Shigella and Klebsiella species.

tested for oxidase reactivity. Oxidase negative isolates were then subjected to appropriate biochemical test screens consisting of triple sugar iron (TSI), lysine-iron agar (LIA), or motility-indole-ornithine medium (MIO). Presumptive Salmonella and Shigella isolates were confirmed with commercially-available polyvalent antisera (Figure 1).

Klebsiella--Appropriate aliquots of processed sludge were dilution plated in triplicate to eosin methylene blue (EMB) agar as shown in Figure 1. Mucoid colonies were isolated, subcultured, and tested for an oxidase negative reaction. Suspect Klebsiella isolates were verified by typical biochemical reactions in TSI or MIO media.

Fluorescent Pseudomonads--Pseudomonas agar F, Pseudomonas agar P, and centrimide agar all allowed the detection of fluorescent pseudomonads in sludge samples. However, centrimide agar, being a more selective medium, resulted in more discrete fluorescent colonies. Confluent growth of other organisms on Pseudomonas agars F and P made counting of colony-forming units unreliable.

Appropriate dilutions were spread plated in triplicate on centrimide agar. Plates were incubated at 37°C for 24 hours. To allow optimal pigment production, plates were then exposed to fluorescent light for 18-24 hours at room temperature prior to counting under long-wave light.

Staphylococcus--Appropriate dilutions of processed sludge were spread plated directly onto plates of mannitol salt agar. Plates were then incubated at 37°C for 24-30 hours. Typical colonies showing a yellow zone due to mannitol fermentation were counted and subcultured for eventual microscopic observation of Gram-positive cocci. All isolates shown to be Gram-positive cocci were then tested for coagulase activity.

Mycobacteria--Methods for the enumeration of mycobacteria in sludge were modified in an attempt to suppress overgrowth of indigenous saprophytic organisms. Nine mL of sludge was mixed with 1 mL of diluted Zephirin® to give a final concentration of 1600 ppm benzalkonium chloride and vortex mixed for 30 seconds. After an extended treatment period of 30-40 minutes, samples were dilution plated onto Middlebrooks 7H11 agar plus OADC enrichment modified by the addition of 3 µg/mL amphotericin B (Fungizone®). Plates were incubated at 37°C in an atmosphere of 5% CO_2-in-air and observed over a period of 4 weeks for growth. Isolated colonies were stained (Ziehl-Neelsen) and observed for acid-fast bacilli. Additionally, all nonchromagens were subcultured onto Lowenstein-Jensen tubed media and subsequently tested for niacin production, a distinguishing characteristic of M. tuberculosis.

Clostridium perfringens--A total of four media were evaluated for the quantitative recovery of Clostridium perfringens from sludge. Two media, litmus milk and differential reinforced clostridia media (DRCM), were employed in a multiple tube (MPN) procedure, and two media, sulphadiazine-polymixin B-sulfite agar (SPS) and Bonde's media, were used in a direct plate enumeration.

After vortex mixing, one portion of the sludge sample was inoculated into test media for enumeration of vegetative clostridia. Another aliquot of sludge was heat shocked by holding at 80°C for 30 minutes prior to inoculation in order to assay for sporulated clostridia. Each sample then was diluted in phosphate-buffered saline. A series of five tubes was inoculated at each dilution in the MPN determinations, while three plates were used at each dilution in the pour plating procedures. All media were incubated at 35°C for 24-72 hours.

After appropriate incubation, confirmation tests were run on positive samples. Wet mounts and stains were made and observed for the presence of nonmotile, encapsulated Gram-positive rods. Additionally, a loopful from each DRCM tube showing a positive reaction was transferred to litmus milk and subsequently examined for typical stormy fermentation.

During preliminary testing, no recoveries of clostridia were made on Bonde's agar. Results from sludge samples for the other test media are compiled in Table 4. All three test media resulted in positive isolations of C. perfringens.

Selection of a routine procedure involved further considerations based on handling and reproducibility of results. The use of SPS agar in a pour plate required special handling to insure anaerobiosis; additionally, contaminating fungi posed a continuing problem. Litmus milk as a primary isolation media led to equivocal results because of the rather subjective judgment of "stormy fermentation." Many tubes had to be examined by the staining procedure; therefore, the overall confirmation rate was moderate (76% in these studies).

Differential reinforced clostridia medium (DRCM) was selected as the medium of choice in a multiple tube, MPN procedure as described previously. A positive test was easily discerned by the medium turning black; sampling for wet mounts was easier (no material blockage that occurs with curdled milk), and the overall confirmation rate was higher (92%).

Gram-negative Enteric Bacteria--A screening procedure (Figure 2) was outlined to allow the quantitative enumeration of both oxidase-negative and oxidase-positive enteric bacteria including all members of the family Enterobacteriaceae. Processed sludge samples were diluted appropriately in sterile phosphate-buffered saline and spread plated over three plates per dilution. MacConkey agar and xylose-lysine-desoxycholate (XLD) agar were used as the primary plating medium. After incubation at 37°C for 24 hours, all colonies were counted, isolated, and subcultured from each plating medium at a dilution having a total of approximately 100 colonies. Discrete colonies were streaked onto quadrants on MacConkey agar plates to allow growth and confirmation of purity.

Subsequent identification involved oxidase testing and use of API 20E® biochemical test strips. The API 20E® system consists of

TABLE 4. RECOVERY OF CLOSTRIDIA FROM SLUDGE SAMPLES

Sample Source	Litmus Milk MPN/100 mL		DCRM MPN/100 mL		SPS agar cfu/100 mL	
	±25°C	80°C	±25°C	80°C	±25°C	80°C
Digested sludge, San Antonio	3.4×10^6	2.6×10^6	1.5×10^6	7.0×10^5	1.6×10^6	4.3×10^5
Wasted sludge, Kerrville	1.7×10^6	5.4×10^7	3.4×10^6	1.6×10^7	4.8×10^6	9.1×10^6

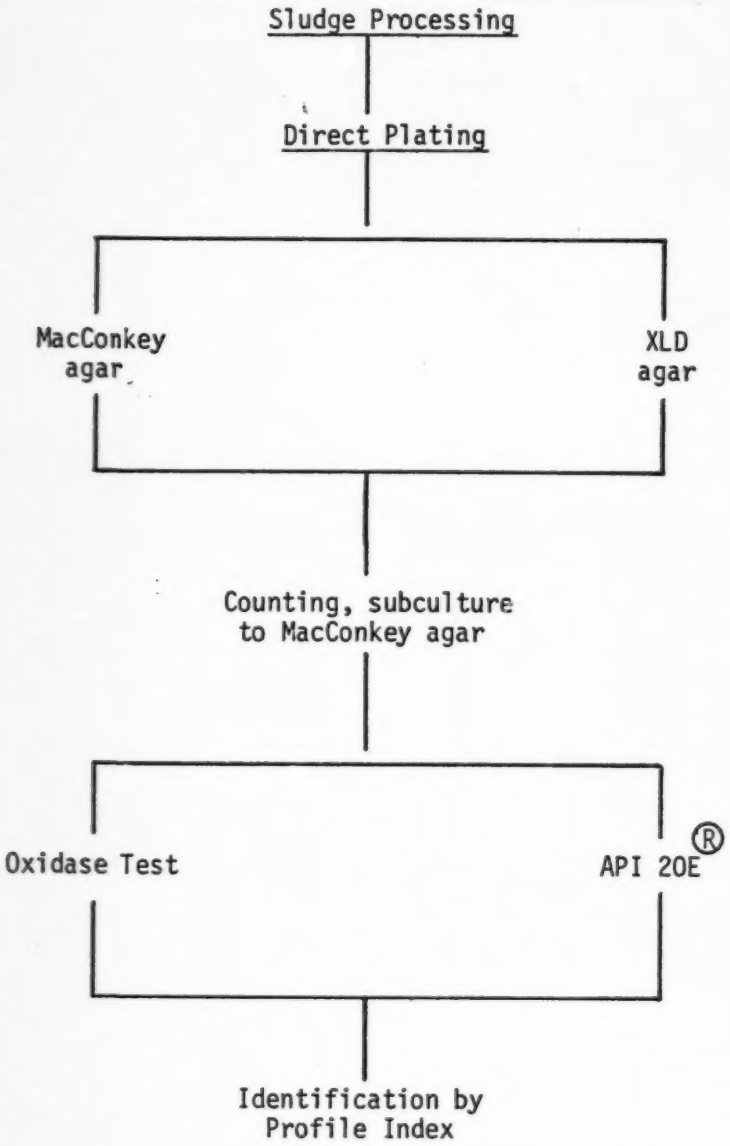

Figure 2. Schematic for isolation of enteric bacteria.

a preset battery of 20 microtubes which allow the performance of 22 biochemical tests for the identification of 49 species/subspecies of Enterobacteriaceae and 38 group/species of other Gram-negative bacteria.

Virology--

Bacteriophage--To assure a uniform sample dispersion, sludge samples were processed by vortex mixing for 2 minutes in the presence of 3-mm glass beads which had been treated with Siliclad®. Direct assay of the processed samples on Escherichia coli K-13 resulted in the recovery of 20% more plaque-forming units (pfu) than from the same unprocessed sludge sample.

Detection of indigenous coliphage was done by assaying appropriate dilutions of processed sludge using an 18-hour aerated broth culture of E. coli K-13 as host. In all cases, bacteriophage were detected by plating the sample at final dilutions ranging from 1:10 to 1:200. No bacteriostatic effects toward the host organism were observed using this approach. Classical soft agar overlay procedures using tryptose phosphate agar (Difco) was used. Plates were incubated at 35°C for eight hours and counted. This abbreviated incubation period was necessary to avoid overgrowth of indigenous sludge bacteria. A maximum of ten plates was used for the 1:10 dilution of sample, and five plates were used at each subsequent serial dilution.

Enteroviruses--Published literature on methodology for viral recovery from sludge was considered in designing a protocol for the isolation of viruses from sludge. A limited number of approaches have been proposed for viral elution from sludge solids followed by viral reconcentration into a volume suitable for in vitro assay. Critical parameters for virus recovery are: mode of solids' disruption, elution medium employed, and efficiency of viral concentration in the presence of toxic and/or interfering components.

Wellings et al. (6) compared indigenous viral isolations from identical sludge samples using sonication, mechanical stirring, and fluorocarbon extraction of sludges in 3% beef extract. Each technique resulted in positive isolations, although no one treatment method resulted in consistently positive results. Based on their field experiments, these researchers recommended that sonication methodology be included as a simple means of sludge dispersal for releasing solids-associated viruses.

Moore and co-workers (7) reported the recovery of indigenous viruses from primary and digested sludges using blender homogenization. Multiple (repetitive) homogenization-elution of the same sludge sample (recovering the solids after each step) demonstrated that of the total number of viruses recovered through either two or three elutions, generally 90% of those detected were released by the first homogenization-elution.

Sonication and mechanical mixing either by magnetic stirring or shaker table mixing were compared by Hurst et al. (8). Their recommendations

were that a glycine buffer, pH 11.5, be used to elute viruses from sludge solids by vigorous magnetic stirring for 30 seconds. After removing solids by centrifugation, the pH of the sample was lowered to 3.5. The resulting floc containing attached viruses was recovered by centrifugation and resuspended in fetal calf serum to elute concentrated viruses. After an additional centrifugation and filtration, the sample was assayed.

Glass et al. (9) selected sludge disruption by sonication at 170W for 2 minutes at ambient pH using 3% beef extract as an eluting medium. Beef extract eluates were concentrated using the classic organic flocculation technique (10). Sample toxicity of concentrated volumes was alleviated by extraction with dithiozone in chloroform prior to plating.

Most quantitative work [specifically Hurst (7) and Glass (8)] has been based on the recovery of exogenous viruses which have been passively mixed with sludge samples. This mode of superficial attachment of viruses to sludge solids bears little resemblance to real-world samples where viruses may be embedded in a solids matrix. The intent of the experimentation described below was to optimize a procedure for the recovery of indigenous enteric viruses from domestic wastewater sludges. Samples were obtained from the Rilling Road Wastewater Treatment Facility in San Antonio, Texas as either primary or anaerobically-digested sludges.

Several methods utilizing a variety of elution and reconcentration procedures were evaluated in parallel. In all cases, equal volumes of liquid sludge and suspending medium were combined prior to sample processing for viral elution. Three methods of dispersing sludge solids with a concomitant release of indigenous viruses were compared. They were blender homogenization for 3 minutes, mixing on a magnetic stirrer for 30-60 seconds, and sonication (60W output for 5 minutes, Branson Sonifier Model 200).

As indicated in Table 5, the choice of suspending media for the primary elution of viruses from sludge solids dictated the reconcentration procedures used. A classical organic flocculation procedure as described by Wellings et al. (6) was adopted for viral concentration from beef extract and glycine buffer eluates. After removing the eluted sludge solids by centrifugation at 3,000 x g for 5 minutes, the pH of the supernatant was dropped to 3.5 by addition of HCl. The resultant floc was mixed for 30 minutes to allow viral attachment and then recovered by centrifugation at 6,000 x g for 5 minutes. Initially, the pellet was resuspended in a 0.15M $NaHPO_4$ buffer; however, problems involving sample toxicity toward cell monolayers necessitated the use of calf serum as an alternative resuspending medium. After this final elution, the sample was centrifuged at approximately 7,500 x g for 15 minutes. The supernatant was recovered for assay.

TABLE 5. SYSTEMS FOR THE RECOVERY OF VIRUSES FROM SLUDGE

Eluting Medium	Reconcentration Procedure
Beef extract, pH 9	Organic flocculation
Distilled water	Bentonite concentration
Glycine buffer, pH 11	Organic flocculation

Viral recovery from distilled water eluates was accomplished using a bentonite concentration procedure. After the removal of sludge solids following elution (see above), 200 mg/L bentonite clay was added along with $CaCl_2$ to a final concentration of 0.02M. The sample was adjusted to pH 6.0 and mixed for 30 minutes. The bentonite solids were recovered by centrifugation at approximately 3,000 x g for 5 minutes and eluted with tryptose phosphate broth. The final concentrated volume was obtained after centrifugation at approximately 7,500 x g for 30 minutes to remove bentonite.

Sporadic sample toxicity resulted in the failure of several early experiments. In almost all cases, attempts to plate undiluted sludge concentrates resulted in dead cells within 24-48 hours post-inoculation. To alleviate toxicity, two approaches have proven feasible. Dilution of the sample in an equal volume of phosphate-buffered saline allowed completion of a successful assay in most cases. However, this remedy increased the plating volume and essentially contradicted the goal of concentration. Alternatively, overnight dialysis (molecular weight cutoff: 6,000-8,000) of concentrated samples against sterile phosphate-buffered saline at 4°C also counteracted toxicity problems. Subsequent work utilized a final step of sample dialysis prior to plating on cell monolayers.

Results presented in Table 6 illustrate the dependence of viral recovery on the solids' dispersal techniques used. In these experiments, a sludge sample was split into three equal volumes and mixed with the specified eluant prior to handling by one of the three methods listed. The data suggested that the efficiency of viral release from sludge for any given elution medium depended on the mode of solids' disruption. In many cases it was observed that magnetic mixing did not result in a visibly sufficient dispersion of solids; this was particularly noticeable in the case of primary sludges. Highest viral recoveries with beef extract were obtained by sludge sonication while blender homogenization was optimal for distilled water. The case of glycine buffer was less definitive, although blender homogenization appeared to be effective.

TABLE 6. VIRAL RECOVERY FROM SLUDGE AS A FUNCTION OF PROCESSING TECHNIQUE

Eluting Medium	Sludge Type	Magnetic Mixing (30-60 seconds) pfu/g TSS*	% Max.	Blender Homogenization (3 min) pfu/g TSS*	% Max.	Sonication (60W - 5 min) pfu/g TSS*	% Max.
Distilled Water†	Primary	5.4	51	10.5	100	8.2	78
	Digested	8.2	17	47	100	31	66
	Digested	6.6	29	23	100	6.2	27
Glycine Buffer, pH 11‡	Primary	28	67	42	100	40	95
	Digested	1.0	37	2.7	100	Not Done	
	Digested	10.2	100	2.6	26	Not Done	
Beef Extract, pH 9‡	Primary	2.8	6	35	73	48	100
	Primary	0.5	2	13	57	23	100
	Digested	2.6	96	0.5	20	2.7	100

* Assayed on HeLa cell monolayers.
† All samples concentrated with bentonite as described.
‡ All samples concentrated by organic flocculation as described.

After further preliminary testing, the use of high pH glycine buffer was discontinued because of the large amount of organic matter released from sludges under such conditions. In some sludges, it appeared also that indigenous viruses were not stable especially toward sonication at high pH. During several comparative trials, indigenous virus levels detected using the glycine buffer (pH 11) ranged from 2% to 20% of the maximal numbers observed with the other eluting media.

Comparative viral recoveries of the remaining two elution-concentration methods as well as cell line comparisons are presented in Table 7. Both concentration techniques resulted in virtually identical recoveries of indigenous viruses. Of the cell lines tested, only HeLa, BGM, and HEp-2 cells showed recovery of plaque-forming units (pfu). Neither L-cells nor BS-C-1 cells tolerated assay conditions; inoculation of dialysed as well as diluted samples resulted in cell death. The greatest number of viruses as measured by pfu were detected on HeLa and HEp-2 monolayers using 3-day and 5-day counts.

Subsequently, an additional series of experiments was completed on the beef extract-organic flocculation procedure. More indigenous viruses could be recovered from primary and anaerobically-digested sludges (Rilling Road Treatment Facility) at beef extract concentrations below 3%. Representative results compiled in Table 8 suggest that excess protein interfered with rather than enhanced viral concentration with no beef extract and 1% beef extract resulting in comparable recoveries. Thus, the amount of beef extract used to elute samples was decreased from 3% as recommended in the literature to a final concentration of 1% providing some input of soluble organics. Subsequent handling was as described previously for beef extact elution and organic flocculation.

TABLE 8. RECOVERY OF INDIGENOUS VIRUSES FROM PRIMARY SLUDGE BY ORGANIC FLOCCULATION

Beef Extract, Final Concentration (%)	Virus Recovered (pfu/g TSS)
0	50
1	46
2	29
3	29

Viral enumeration was done by dividing the sample between two cell lines, HeLa and HEp-2. Only one-half of a sample was assayed at a

TABLE 7. INDIGENOUS VIRUSES DETECTED IN PRIMARY SLUDGE

Elution--Concentration Procedure	Viral Recovery, pfu/g TSS				
	HeLa		BGM	HEp-2	
	3d	5d	3d	3d	5d
SAMPLE 1					
Beef Extract--Organic flocculation	56	71	5.6	*	*
Distilled Water--Bentonite	77	92	4.0	*	*
SAMPLE 2					
Beef Extract--Organic flocculation	51	80	9.6	35	51
Distilled Water--Bentonite	48	74	5.1	35	61

* Assay not done.

time with the remaining volume held at -76°C. The frozen volume served as a safeguard against loss of an entire sample due to toxicity or contamination. Secondly, the remaining sample provided confirmation of virus recovery obtained with the first half sample assayed.

Assays were done by inoculating 1.0-mL samples on cell monolayers grown on 100 mm culture dishes. After a 1-hour attachment period, infected plates were washed with a Hank's balanced salt solution (HBSS) containing penicillin G (500 units/mL) and streptomycin (250 µg/mL). The agar-based overlay media consisted of Eagle's minimal essential medium containing 8% calf serum, 100 units of penicillin G per mL, 50 µg of streptomycin per mL, 25 µg of Gentamicin® per mL, and 0.5 µg of Fungizone® per mL. Two days post-inoculation, a second agar overlay containing 30 µg/mL neutral red was applied to the plates. At 3 days and 5 days after infection, developing plaques were scored and recorded. Viral numbers were reported as pfu per gram of total suspended solids.

During the methods evaluation and preliminary site evaluation phases of this project, both the beef extract-organic flocculation and the distilled water-bentonite procedures were utilized in parallel on digested sludges. Viral recoveries were comparable with either approach; however, the bentonite concentration procedure resulted in a smaller plating volume. Results reported for the site evaluation sludge samples reflect distilled water elution-bentonite concentration with the exception of the St. Petersburg, Florida, sample collected on March 5, 1979. In this instance, a laboratory accident during the final centrifugation resulted in the loss of the bentonite sample. Subsequent sludge samples collected in conjunction with the aerosol sampling program were handled by the distilled water elution-bentonite concentration procedure.

Methods Evaluation--

A microbiological screen on primary sludge and anaerobically-digested sludge from the San Antonio Rilling Road Wastewater Treatment Facility was completed during the last two weeks of August 1978. All proposed methodologies were tested during this screen. The purposes of this phase of methods development were to gain practical experience in sludge handling and to assess workload problems posed by simultaneous testing. Results from the screen are presented in Table 9 as number of organisms per gram of total suspended solids.

As in previous microbial screens of wastewater samples, the need to quantitate gram-negative enteric bacterial species dictated that only those organisms present in numbers comparable to the most predominant species were detected. Specifically, in the sludge screen reported in Table 9, the prevalence of Aeromonas hydrophilia in primary sludge at 5.8×10^7 cfu/g limited the identification of other enteric bacteria to those whose numbers exceeded approximately 10^6 cfu/g.

TABLE 9. MICROBIOLOGICAL SCREEN, RILLING ROAD WASTEWATER TREATMENT FACILITY, SAN ANTONIO

	Primary Sludge	Digested Sludge
Bacteria (cfu/g)		
Standard Plate Count	3.3×10^9	8.7×10^8
Total coliform	1.3×10^7	2.6×10^7
Fecal coliform	2.6×10^7	3.3×10^6
Fecal streptococci	7.3×10^6	1.1×10^6
Fluorescent pseudomonads	9.6×10^5	1.3×10^5
Staphylococcus sp.	2.5×10^4	2.2×10^4
Clostridium perfringens (MPN/g)		
-Δ (vegetative)	4.2×10^7	3.1×10^7
+Δ (sporulated)	1.8×10^7	7.3×10^6
Salmonella sp.	≥ 38	≥ 6.7
Shigella sp.	≤ 3.8	≤ 2.7
Klebsiella sp.	1.6×10^7	2.7×10^6
Mycobacteria sp.	1.0×10^9	1.0×10^8
Citrobacter freundii	2.5×10^6	None Detected
Escherichia coli	1.3×10^6	1.3×10^6
Klebsiella oxytoca	1.3×10^6	6.7×10^5
Klebsiella pneumoniae	2.5×10^6	None Detected
Enterobacter cloacae	6.5×10^6	6.7×10^6
Aeromonas hydrophilia	5.8×10^7	3.1×10^7
Virus (pfu/g)		
Coliphage	1.7×10^5	7.3×10^3
Enteroviruses, HeLa (3d)	2.6×10^1	1.0×10^1

The completion of tests for bacteria and viruses in a single sludge sample as outlined required two weeks. During this time all laboratory testing and data evaluation were finalized with the exception of Mycobacteria sp. The extended incubation period for these organisms dictated that 5-6 weeks were required for their enumeration.

Trace Metal Analysis--

All metals except mercury were analyzed by first digesting a representative sludge sample followed by analysis on either a Perkin Elmer Model 403 or Model 560 Atomic Absorption Spectrophotometer. A 200-mL aliquot of each sample was digested with redistilled nitric acid for one hour at 150°C. The samples were then digested an additional two hours after adding hydrochloric acid and more redistilled nitric acid. After concentrating the sample to less than 50 mL, the digest was centrifuged, filtered, and adjusted to a 50-mL final volume. Analyses were performed using an atomic absorption spectrophotometer in the flame mode.

For mercury determination, a 10-mL sample was digested in a reaction vessel at 100°C with 5 mL Hg-free nitric acid, 5 mL sulfuric acid, and potassium permanganate. After digestion and cooling, 5 mL of 12% hydroxylamine hydrochloride and 5 mL of 10% stannous chloride were added to the sample. Mercury content was then immediately determined by the cold vapor technique using a Laboratory Data Control UV monitor.

For purposes of quality control in the trace metal analyses, an aliquot of sludge was spiked with each metal at a level twice that of the metal concentration value. Recoveries of metals from spiked samples varied between 76% for Cd and 135% for Mo. Results of samples were adjusted for recovery efficiency. Additionally, replicate analyses were conducted on a random selection of samples.

Pesticides and PCB's Analysis--

Sludge samples were extracted and analyzed by a method that was simultaneously developed on EPA Contract 68-03-2606, "Method Development for Determination of Polychlorinated Hydrocarbons in Municipal Sludge" (10). In this method, 20 g of homogenized sample were mixed with saturated sodium chloride solution and extracted three times with 60 mL of extracting solvent (83% hexane, 15% methylene chloride, 2% acetone). Sample extracts were concentrated in a Kuderna-Danish apparatus and fractionated on a Bio-Beads column. The three fractions were concentrated in hexane to a 5 mL final volume and analyzed on a Hewlett Packard 5700A Gas Chromatograph equipped with an electron capture detector. The pesticides and PCB's identified were compared to computer-measured retention time of standards and quantified by peak height measurements. No GC/MS confirmation of these tentatively identified compounds was performed.

An aliquot of sludge was spiked at levels ranging from 1 mg/g to 20 mg/g with all pesticide compounds (except toxaphene) and one of the PCB's (AR 1260). Recoveries ranged from 49% for DDD to 106% for endrin. Detection limits and those compounds identified by relative

retention times were adjusted to reflect recovery efficiencies as determined from the spiked samples.

Microbiological Assay of Aerosol Samples

Indicator Organisms--

Assays of the aerosol samples for total coliform, fecal coliform, and fecal streptococci were conducted by the appropriate membrane filter procedure described in Standard Methods, 14th edition. M-Enterococcus agar was substituted for KF streptococcus agar. Results were reported as cfu/100 mL.

Standard Plate Count--

The methods employed for the analysis of aerosol samples were identical to those employed for the analysis of the sludge characterization samples. Results were reported as cfu/mL of aerosol sampler fluid.

Mycobacteria--

To improve detection sensitivity, a concentration procedure was used to isolate mycobacteria from aerosol sampler fluid. A 50-mL volume of Zephiran®-treated sample (3200 ppm benzalkonium chloride) was centrifuged at approximately 3000 x g for 15 minutes. The supernatant was discarded, the pellet was resuspended in 1 mL of sterile saline, and this volume was plated as previously described.

Salmonella--

Enrichment procedures for Salmonella species were used to quantitate (by range) indigenous Salmonella in aerosol samples. Aliquots of sample (2.5 mL and 25 mL) were added to selenite broth and incubated for 24 hours at 37°C. Subsequent handling was as described for Salmonella in sludge.

Bacteriophage--

A 25-30 mL volume of BHI plus 0.1% Tween® was plated as described for the sludge characterization samples.

Enterovirus Aerosol Study

The special virus aerosol study employed procedures developed in conjunction with the Pleasanton study [DAMD 17-75-C-5072, "Evaluation of Health Effects Associated with the Application of Wastewater to Land" (3)] and described by Moore et al. (12). These methods involve two stages, field concentration and assay, each of which is described below.

Field Concentration--

The classical two-phase polymer concentration procedure was used. Appropriate volumes of BHI with 0.1% Tween 80® were mixed with sodium dextran sulfate, polyethylene glycol (PEG 6000), and sodium chloride to final concentrations of 0.2%, 6.45%, and 0.15M, respectively.

The total volume was shaken vigorously in a separatory funnel and held at 4°C for 18 hours to permit adequate phase separation. The lower phase and interphase volumes were collected and diluted (1:3) with HBSS with appropriate antibiotics. The samples were shipped at 4°C to the laboratory for assay.

Viral Assay--

The total volume of the diluted lower phase and interphase were assayed both on HeLa and HEp-2 cell monolayers. Due to the high incidence of fungi in the concentrated aerosol samples, they were diluted further (1:8) using HBSS containing 1 µg/mL Fungizone®. The sample was mixed for 10 minutes and then centrifuged at 5000 x g for 20 minutes prior to inoculating the cell monolayers. After a 60-minute attachment period, the inoculum was removed by aspiration and the infected monolayers rinsed with HBSS containing penicillin and streptomycin as described for sludge characterization samples. The agar overlay was modified by increasing the concentration of Fungizone® to 1.5 µg/mL. Plates were incubated and subsequently stained as previously described.

Chemical and Physical Analysis of Sludges

Procedures outlined in Standard Methods, 14th edition, for the determination of total nonfilterable residue at 103-105°C and volatile residue at 550°C were used for the total suspended solids and volatile suspended solids measurements, respectively.

AEROSOL SAMPLING PROTOCOLS

Microbiological Aerosol Runs

The objective of the microbiological aerosol runs was to measure the microorganism levels downwind of two procedures used for land application of liquid sludges: tank truck application and spray gun application. This was accomplished using eight high-volume air samplers (Litton Model M) in an array based on two separate protocols determined from the characteristics of the sites.

Truck Sites--

At the tank truck sites, the objective was to operate the samplers as closely as possible to the fields being sprayed. The assumption at these sites was that any airborne levels of microorganisms would be so low that sensitivity in the analyses would be a problem. Rather than obtain samples at two different distances for modeling purposes, the emphasis was placed on obtaining good data close to the source. For the tank truck sites, two samplers were paired upwind while the six downwind samplers were deployed as two trios roughly equidistant from the path of travel of the truck (as indicated in Table 10). After each run the samples at each position were pooled to provide a total of three samples for microbiological analysis. The two downwind locations were established 30-40 m apart as close to the spraying operation as feasible given wind direction and field condition. The samplers at both the upwind and downwind locations were placed one meter apart.

TABLE 10. SAMPLER ARRAY FOR MICROBIOLOGICAL AEROSOL RUNS

Sample Designation	Aerosol Source Sampled	Location	No. of Samplers	Sample Processing
TANK TRUCK SITES				
Upwind	Background	Upwind of all sludge aerosols	2	Pooled
Downwind 1	Tank truck aerosol	≈10 to 30 m downwind of truck traverse	3	Pooled
Downwind 2	Tank truck	≈10 to 30 m downwind of truck traverse	3	Pooled
SPRAY GUN SITES				
Upwind	Background	Upwind of all sludge aerosols	2	Pooled
50 m Left	Spray gun aerosol	40 to 60 m downwind of wetline of aerosol source	2	Pooled
50 m Right	Spray gun aerosol	40 to 60 m downwind of wetline of aerosol source	2	Pooled
100 m	Spray gun aerosol	80 to 110 m downwind of wetline of aerosol source	2	Pooled

Wind direction and portion of the field being irrigated required the samplers to be moved between segments of a run. Wind direction and speed were measured at each of the downwind sampling positions by a meteorological station positioned with the samplers. A typical sampling arrangement is shown in Figure 3.

Spray Sites--

At the spray irrigation sites, it was known that there would be aerosols generated, and the objectives were to measure downwind concentrations of microbiological constituents in the aerosol, estimate the impact and decay factors, and compare these to sites practicing spray application of wastewater. To accomplish these three objectives, four aerosol sampling stations were operated on each run, one upwind to determine background levels, two at 50 meters from the wet line of the spray source with a separation of 10 to 15 m and one at 100 meters from the wet line edge. Two samplers were operated at each location to increase the sensitivity of the analyses and to reduce variablility due to differences between samplers. The collection media at each location were pooled after each run and sent for analysis.

At the two downwind distances and one upwind location, high-volume particulate samplers were operated to measure the concentration of particulate matter in the air. Since liquid sludge is approximately 5% solids, aerosolization of the sludge would imply that particulates arising from the spray would be carried downwind. In addition, it was assumed that the microbiological constituents would exist primarily in the solids phase of the sample, and the particulate aerosol matter generated by the source would be of special interest in studying the distribution of microbiological components of the sludge. Meteorological stations were placed at the 50- and 100-m sites to provide wind direction and speed information. An example of the sampling configuration for the spray sites is shown in Figure 4.

Meteorological Parameters--

Proper meteorological conditions must prevail for an aerosol run to be attempted. These included suitable and consistent wind speed and wind direction accompanied by a lack of precipitation. These conditions were monitored visually and by observing wind speed and wind direction with the MRI portable weather station. No aerosol runs were attempted during periods of rainfall since moisture causes high voltage arcing problems in the Model M samplers. A few runs were attempted during periods of high humidity with little or no arcing problem.

Once the prevailing wind direction was determined, sampler positions were assigned and set-up commenced as described earlier in this section. Before initiating the run, meteorological conditions were again evaluated for any changes in the prevailing wind direction and direction variability. If conditions were satisfactory, the run was started and completed according to the procedure given in Appendix C.

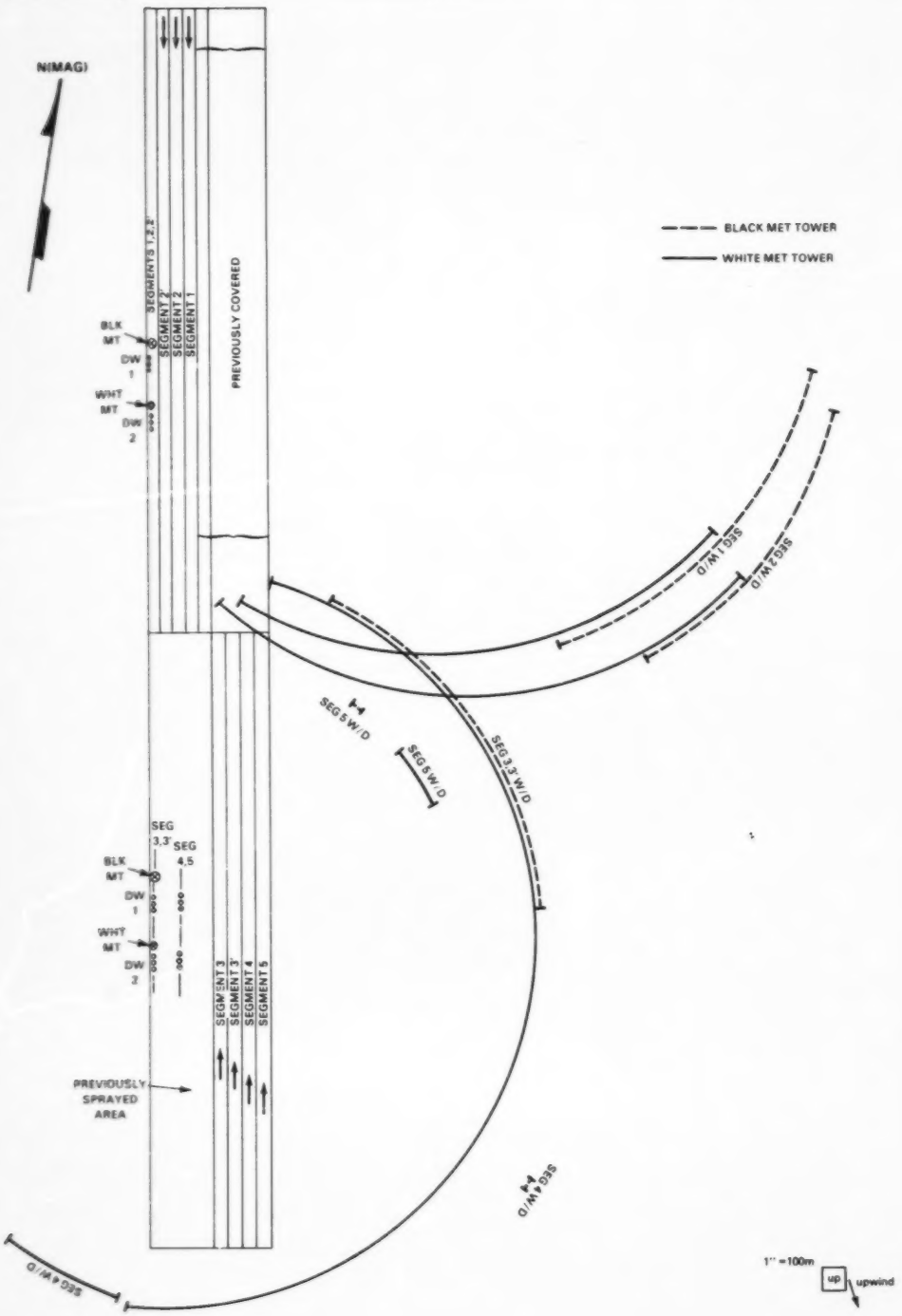

Figure 3. Sampling configuration at tank truck sites

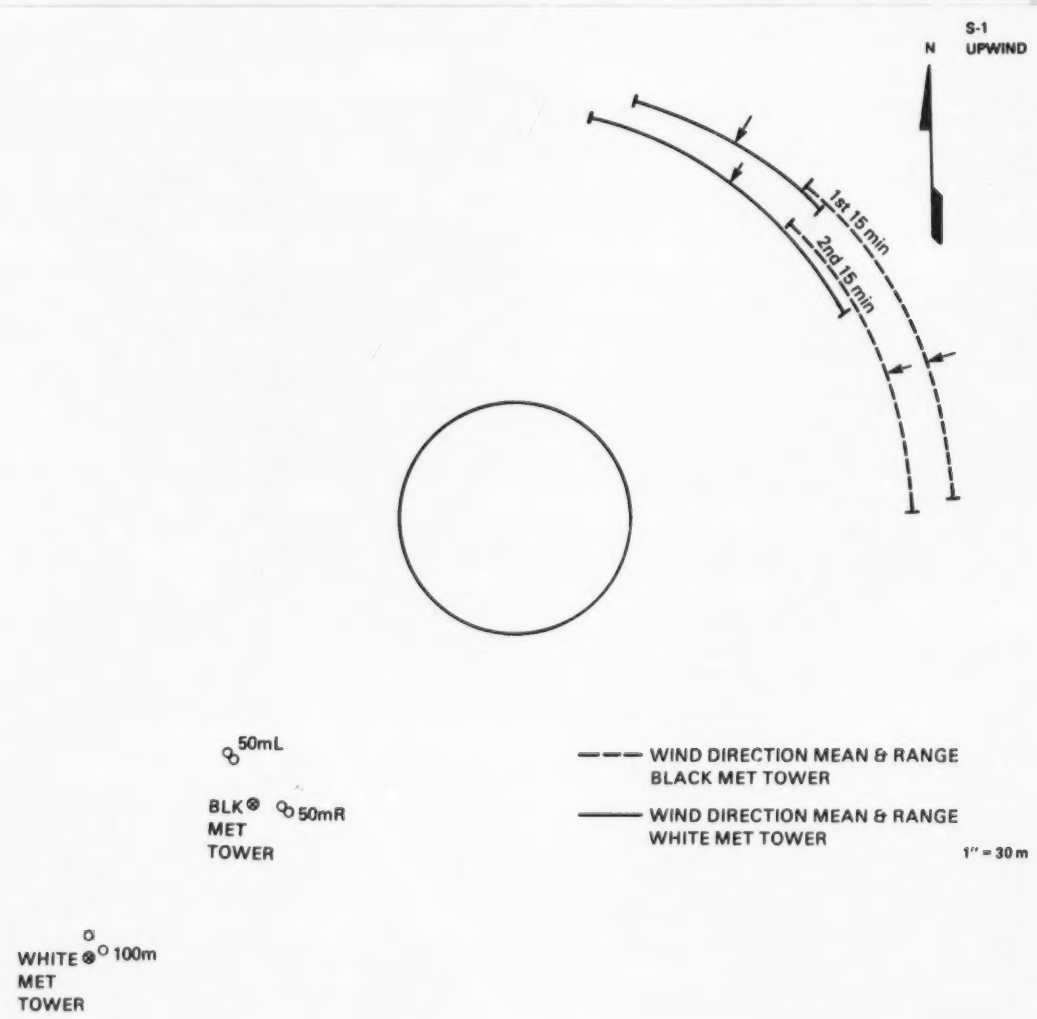

Figure 4. Sampling configuration at spray sites

At the end of the run, wind direction data from the two meteorological stations and other run parameters were evaluated according to the following criteria established as guidelines for a good aerosol run:

1) average wind direction for the segments of the run was no more than 30° from the wind direction angle used for sampler set-up;

2) the extremes of wind direction (excluding outlier spikes) was no more than 60° from the wind direction average;

3) at least one sampler at each sampling location completed the run;

4) no significant precipitation occurred during the run.

If these criteria were satisfied, samples were pooled, labeled and stored at 4°C until shipment that evening. Prior to shipment, samples were packed in an insulated mailer with a frozen Kool Pac® and shipped via counter-to-counter parcel service of a scheduled commercial airline. With this arrangement, the elapsed time between sample collection and arrival in the CART laboratories was consistently less than 24 hours.

Enterovirus Aerosol Run

The objective of the virus study was to determine the enterovirus aerosol concentrations just downwind of a sludge aerosol source using specialized sampling methodologies and concentration techniques. First it was necessary to determine if measurement of enteric viruses in aerosols downwind of a sludge spray gun was practical with state-of-the-art sampling procedures and equipment. Since the levels of viruses in sludges are relatively low, though appear to be within an order of magnitude of wastewater virus levels, it is difficult to detect aerosolized viruses even with the level of sensitivity available with the high-volume samplers. However, by utilizing all available samplers in tandem operation and concentrating the collection fluid, it is possible to achieve limited sensitivity for virus collection. By correlating results obtained for spray aerosols from wastewaters to the levels of viruses in sludges, it appeared that a minimum of 1000 m^3 of air must be sampled to warrant conducting an enterovirus aerosol run.

To meet this requirement, nine high-volume Model M samplers were operated simultaneously for six consecutive 30-minute sampling periods in close proximity to each other (1.5 m apart) at 50 m (164 ft) downwind of a sludge aerosol source. Since sampling time would be a minimum of three hours, the St. Petersburg site with continuous spraying was chosen as the site for attempting the virus run.

After placing the samplers in the proper downwind location, the virus run was started in the same manner as a regular aerosol run.

However, after each 30-minute sampling period, the sample containers with 100 mL of BHI media were capped, and a new 30-minute sampling period was initiated with a new bottle of BHI medium. At the end of the virus run, all BHI collected was transported to the State of Florida Epidemiology Laboratory, Tampa, Florida for concentration. Field concentration of the BHI was performed by SwRI personnel utilizing the procedure described in the subsection "Enterovirus Aerosol Study."

During the virus run, a one-liter grab sample was collected every 30-minute sampling period from the irrigation system supplying the spray gun generating the aerosol. The grab samples were composited, cooled, and an aliquot transferred to two one-liter bottles prior to shipment to the CART laboratories.

Some variation in wind direction could be tolerated during a 30-minute run period since the samplers were located only 50 m from the broad aerosol source provided by the rotating spray gun. Some shifting in wind direction over the duration of the enterovirus aerosol run was also tolerable since samplers could be relocated between the 30-minute run periods.

Problems Associated With Aerosol Sampling

Collection of aerosol samples at the tank truck sites presented three unique problems. The first was the task of obtaining aerosol samples for a moving source (the truck) with the necessary sampling equipment in a stationary position and the distance between sampler and aerosol source continually changing. Since it was impossible to sample at a constant distance from the source, the two downwind sampling locations were placed in a position on the downwind side of a truck traverse at a perpendicular distance to the traverse of 10 to 30 m (33 to 98 ft). The closest distance of aerosol formation to the samplers was the distance along the wind angle between the source and the sampler.

A second problem developed in obtaining sufficient sampling time since each truckload of 5.7 cubic meters (1,500 gallons) of sludge could be applied in two minutes or less. Consequently, it was necessary to operate samplers only during the period of aerosol generation and then await the next aerosol segment. At the Longmont site, this waiting period was up to one hour due to the distance of the application site from the sewage treatment plant. Since the application site at Cibolo was adjacent to the treatment plant, the average time between truck traverses was 15 minutes. This fact coupled with cooler ambient temperatures during the sampling period perhaps resulted in more valid aerosol data for the Cibolo aerosol runs than the Longmont aerosol runs.

Another problem developed in sampling because the location of the truck traverse was different with each load of sludge applied. As a result, with each pass the distance between samplers and source either increased or decreased by about 6 to 8 m (20 to 26 ft) over

the previous traverse. Therefore, as the number of traverses accumulated, a point was reached where relocating the downwind sampler positions was necessary to maintain a valid sampling distance during subsequent segments required to meet the sample collection time requirement. This moving requirement perhaps contributed to a possible contamination problem observed at the Longmont site caused either by the physical movement of the sampling equipment, the additional time that BHI media remained in the sampler and associated tubing, or a combination of these and other factors.

At the two spray gun sites, the distance between the aerosol source and the high-volume samplers remained fixed for the duration of the runs. Since the time of spraying at the Portland site was determined by the number and size (22.7 m^3 or 6,000 gallons) of sludge truck runs to the disposal site, an aerosol run consisted of sampling two or more truckloads to obtain a minimum sampling time of 30 minutes. On some runs, at least one hour elapsed between actual sampling segments, and at times it was necessary to move sampler locations due to shifts in wind direction. However, at the St. Petersburg site, spraying occurred uninterrupted resulting in a continuously collected aerosol sample. Here the sampling locations could be assigned based on current wind direction, and in all cases the run was initiated and completed prior to any major shifts in wind direction. Based on this condition, it can be inferred that the more valid aerosol samples from a spray gun operation were collected at this site.

SECTION 6

RESULTS

SLUDGE CHARACTERIZATION STUDY

The samples collected from the six sites assessed for suitability for aerosol sampling provide information on typical levels of organic, inorganic, and microbiological components of liquid sludge used in land application. The summary results are presented for each category of analysis.

Organic Analyses

The sludge samples were analyzed for 16 pesticides and 7 formulations of polychlorinated biphenyls (PCB's). Chlordane was the only pesticide detected, being found at the 11 µg/g level in both samples from Cibolo, at 0.21 µg/L in the first sample from Shreveport, and at 16 and 15 µg/g in the two samples from St. Petersburg. Aroclor 1248 was the only PCB formulation detected in the sludge samples, being found at the 26 and 25 µg/g levels in the two samples from Muncie. No pesticides or PCB's were found at either the Longmont or the Portland sites. The results are summarized in Table 11.

Inorganic Analyses

The sludge samples were analyzed for seven trace metals, cadmium, copper, mercury, molybdenum, nickel, lead, and zinc and the results are presented in Table 12. The first sample from Shreveport had low levels of all metals, but its solids content was too low to be considered a true sludge sample and is not considered in the summaries that follow.

The concentration of each metal, except molybdenum, from all eleven samples from the six sites were used to represent typical levels of trace metals in municipal sludge. Molybdenum was below the detection limits in eight of the eleven samples; therefore, no summary was prepared for this metal. The mean concentrations for the metals ranged from 15.7 µg/g for mercury to 9,180 µg/g for zinc. Confidence intervals were calculated for each of the six metals to represent the mean concentration that one could expect in sludge, under the assumption that the six sites studied are representative of what one would expect in municipal sludge. The data summary is shown as Table 13.

TABLE 11. PESTICIDE AND POLYCHLORINATED BIPHENYL (PCB) SCREEN

	Tank Truck Sites						Spray Gun Sites			
	Muncie		Longmont		Cibolo		Portland		Shreveport	St. Petersburg
	10-7-78	2-20-79	10-16-78	11-27-78	2-5-78	3-19-79	10-30-78	12-11-78	11-13-78 1-22-79	1-8-79 3-5-79
Pesticide (μg/g)										
α-BHC	<0.26		<0.37		<0.30		<0.45		<0.14	<0.31
β-BHC	<1.4		<0.51		<0.82		<1.2		<0.33	<0.91
δ-BHC	<1.1		<0.42		<0.64		<0.97		<0.16	<1.2
Heptachlor Epoxide	<5.8		<2.2		<3.3		<5.0		<1.4	<4.9
DDE	<2.7		<2.4		<1.9		<3.0		<0.80	<5.7
DDD	<11		<4.7		<4.5		<11		<2.9	<21
DDT	<1.8		<2.0		<1.2		<3.5		<1.0	<2.3
ε-BHC	<1.4		<0.52		<0.43		<0.60		<0.38	<0.83
Heptachlor	<0.94		<0.42		<0.60		<0.49		<0.32	<0.90
Aldrin	<0.96		<0.44		<0.54		<0.51		<0.24	<1.3
Endosulfan I	<2.5		<0.56		<0.14		<0.67		<0.56	<1.9
Dieldrin	<1.2		<1.3		<1.0		<0.76		<0.30	<2.3
Endrin	<1.8		<2.0		<0.91		<1.1		<1.0	<1.7
Endosulfan II	<0.95		<1.0		<0.66		<1.2		<0.57	<1.2
Chlordane	<19*		<2.8		11	11	<3.2		0.21** <1.5	16 15
Toxaphene	<100*		<16		<9.7		<18		<7.9	<18
Polychlorinated biphenyls (μg/g)										
AR 1016	<1.8		<2.0		<1.2		<2.2		<1.0	<2.2
AR 1221	<4.0		<4.9		<3.0		<5.7		<2.5	<5.6
AR 1232	<4.4		<4.9		<3.0		<5.7		<2.5	<5.6
AR 1242	<2.5		<2.8		<1.7		<3.3		<1.3	<3.1
AR 1248	26	25	<3.9		<2.5		<4.6		<2.0	<4.5
AR 1254	<3.5		<3.9		<2.5		<4.6		<2.0	<4.5
AR 1260	<7.0		<8.0		<4.8		<9.2		<3.9	<9.0

* High minimum detection limit due to PCB background.

** Analyzed as a wastewater sample due to low suspended solids (765 mg/L).

TABLE 12. TRACE METAL SCREEN

	Tank Truck Sites						Spray Gun Sites					
	Muncie		Longmont		Cibolo		Portland		Shreveport		St. Petersburg	
	10-7-79	2-20-79	10-16-78	11-27-78	2-5-79	3-19-79	10-30-78	12-11-78	11-13-78*	1-22-79	1-8-79	3-5-79
Trace metals (μg/g)												
Cadmium (Cd)	33	38	23	16	61	99	180	140	<30	75	63	69
Copper (Cu)	3000	3400	2700	1800	3300	5100	4500	3700	200	1700	8100	7100
Mercury (Hg)	5.5	8.0	6.2	6.7	11	21	19	14	<3	17	45	19
Molybdenum (Mo)	<24	<35	<33	<39	<20	48	40	140	<100	<20	<45	<43
Nickel (Ni)	380	630	160	78	82	73	560	550	<100	99	130	170
Lead (Pb)	3000	3200	720	550	2900	3300	5000	4800	400	2700	2400	2500
Zinc (Zn)	11000	13000	3300	2000	7000	9600	18000	9700	700	5700	9700	12000

* Analyzed as a wastewater sample due to low suspended solids (765 mg/L).

TABLE 13. DATA SUMMARY FOR TRACE METALS IN SLUDGE
(μg/g dry weight)

	Metal					
	Cadmium	Copper	Mercury	Nickel	Lead	Zinc
Maximum	180	8100	45	630	5000	18000
Minimum	16	1700	5.5	73	550	2000
Mean	72.5	4040	15.7	265	2800	9180
St. Deviation	50.5	2040	11.3	220	1400	4550
Confidence Limits						
Upper	106	5410	23.3	413	3740	12200
Lower	38.6	2670	8.10	117	1860	6120

Microbiological Analyses

The 12 sludge samples were analyzed in depth for routine microbiological and physical characteristics, for non-Enterobacteriaceae organisms, and for Enterobacteriaceae organisms. The results of these analyses are shown in Tables 14, 15, and 16, respectively. The most noticeable results from the routine analyses were the high concentrations of Salmonella in the two samples from St. Petersburg, which led to the inclusion of that organism in the aerosol monitoring portion of the study. Staphylococcus sp. were detected in only four of the twelve sites and Shigella sp. in only one. Of the viruses, four of the HEp-2 results were below the detection limit.

Those microorganism groups detected at all sites were used to prepare a data summary as in the case of the trace metal results, and these summaries are shown in Table 17. For the microbiological analyses, the geometric mean and standard deviation were calculated, confidence limits determined in the transformed scale, and confidence limits exponentiated to give the limits shown. Total and volatile suspended solids (TSS and VSS) were summarized by arithmetic mean and standard deviation and were not transformed. The confidence limits represent the upper and lower bounds on the mean concentrations of these parameters in liquid sludge, under the assumption that these sites produce sludge that is representative of sludge in general.

The statistical summary for non-Enterobacteriaceae organisms is shown in Table 18 for the four bacteria detected at all sites, although not necessarily in all samples: Aeromonas hydrophila, Pseudomonas cepacia, Pseudomonas fluorescens, and Pseudomonas putida. The mean concentrations for these oragnisms ranged from 3.1×10^5 to 2.5×10^6, which is roughly the same concentration as fecal coliform. Confidence limits were established as with the routine organisms as an indication of the average concentration expected in liquid sludge.

TABLE 14. BACTERIAL SCREEN SUMMARY— ROUTINE MICROBIOLOGICAL AND PHYSICAL ANALYSES

Sample Source	Tank Truck Sites						Spray Irrigation Sites					
	Muncie, Ind.		Longmont, Co.		Cibolo, Tx.		Portland, Or.		Shreveport, La.		St. Petersburg, Fla.	
Sample No.												
Date Collected	10-07-78	2-20-79	10-16-78	11-27-78	2-05-79	3-19-79	10-30-78	12-11-78	11-13-78	1-22-79	1-8-79	3-5-79
Bacteria (cfu/gm)												
Standard Plate Count	4.8×10^{6}	2.9×10^{6}	7.3×10^{7}	4.8×10^{6}	1.2×10^{6}	7.3×10^{10}	3.6×10^{7}	1.6×10^{7}	8.9×10^{9}	2.2×10^{9}	6.7×10^{8}	4.8×10^{9}
Total Coliform	6.5×10^{6}	1.6×10^{7}	8.8×10^{5}	2.1×10^{5}	5.1×10^{6}	3.9×10^{7}	5.0×10^{5}	1.3×10^{6}	3.6×10^{7}	6.1×10^{7}	1.9×10^{7}	4.0×10^{7}
Fecal Coliform	4.8×10^{5}	3.3×10^{5}	8.8×10^{4}	1.2×10^{4}	3.1×10^{6}	7.8×10^{6}	8.8×10^{4}	1.6×10^{5}	9.1×10^{6}	4.7×10^{6}	5.4×10^{6}	3.6×10^{6}
Fecal streptococci	2.4×10^{5}	1.3×10^{7}	9.2×10^{4}	2.1×10^{5}	6.9×10^{4}	1.2×10^{6}	1.1×10^{5}	6.8×10^{4}	4.1×10^{4}	4.5×10^{5}	4.5×10^{6}	2.3×10^{6}
Fluorescent pseudomonads	6.4×10^{4}	1.3×10^{5}	$<1.3\times10^{3}$(ND)	1.1×10^{5}	2.0×10^{4}	6.3×10^{4}	$<6.6\times10^{2}$(ND)	3.0×10^{5}	1.0×10^{6}	7.1×10^{4}	1.0×10^{5}	3.7×10^{5}
Staphylococcus sp.	$<7.9\times10^{3}$(ND)	$<6.9\times10^{3}$(ND)	$<1.3\times10^{3}$(ND)	$<1.1\times10^{4}$(ND)	$<6.7\times10^{3}$(ND)	$<2.4\times10^{4}$(ND)	$<6.6\times10^{2}$(ND)	$<1.5\times10^{3}$(ND)	2.1×10^{7}	1.2×10^{6}	4.5×10^{4}	8.7×10^{4}
Clostridium perfringens												
-vegetative (MPN/gm)	2.6×10^{7}	8.1×10^{6}	1.8×10^{7}	3.5×10^{6}	3.1×10^{7}	5.9×10^{7}	7.8×10^{6}	2.1×10^{7}	1.6×10^{7}	2.2×10^{7}	1.2×10^{7}	2.0×10^{8}
-sporulated (MPN/gm)	2.6×10^{7}	5.0×10^{6}	5.6×10^{5}	7.7×10^{7}	4.9×10^{6}	1.7×10^{6}	2.2×10^{6}	5.0×10^{5}	2.6×10^{6}	4.7×10^{6}	4.9×10^{6}	1.0×10^{7}
Mycobacteria sp.	4.0×10^{7}	1.2×10^{8}	7.7×10^{7}	$<1.1\times10^{6}$(ND)	4.1×10^{7}	$<8.0\times10^{6}$	8.0×10^{5}	4.5×10^{5}	5.0×10^{8}	2.4×10^{9}	7.7×10^{7}	1.6×10^{8}
Salmonella sp.	2.4454424	>2.1	3.8454438	3.2454432	≥2.0	$\geq2.4\times10^{2}$	2.0454420	4.5454445	1.3×10^{3}<$\leq$<1.3×10^{3}	≥2.0	$\geq4.3\times10^{3}$	$\geq4.3\times10^{2}$
Shigella sp.	$<9.5\times10^{-1}$	<0.8	$<1.3\times10^{0}$	$<1.2\times10^{0}$(ND)	$\geq2.0\times10^{1}$	<1.0	$<8.0\times10^{-1}$	$<1.8\times10^{0}$(ND)	$<5.0\times10^{1}$(ND)	$<7.8\times10^{-1}$	$<1.9\times10^{0}$	<1.7
Klebsiella sp.	8.8×10^{6}	1.6×10^{6}	3.5×10^{5}	1.1×10^{7}	4.7×10^{5}	1.5×10^{7}	3.2×10^{5}	3.2×10^{5}	3.6×10^{6}	1.2×10^{7}	2.9×10^{7}	2.4×10^{6}
Viruses (pfu/gm)												
Bacteriophage	1.1×10^{5}	1.3×10^{5}	5.2×10^{4}	1.1×10^{5}	4.9×10^{6}	1.6×10^{5}	1.7×10^{4}	8.6×10^{3}	2.2×10^{3}	1.4×10^{3}	8.5×10^{4}	2.3×10^{5}
Enteroviruses												
HeLa	1.7×10^{1}	8.5×10^{-1}	6.0×10^{-1}	1.1	5.0×10^{0}	1.0×10^{1}	3.0×10^{-1}	2.3	6.7^{**}	1.2×10^{0}	1.1×10^{0}	4.3×10^{-1}
HEp-2	ND*	$<8.0\times10^{-1}$	4.0×10^{-1}	$<2.2\times10^{-1}$	5.6×10^{-1}	1.2×10^{1}	3.0×10^{-1}	$<4.0\times10^{-1}$	1.4×10^{0}	8.0×10^{-1}	7.6×10^{-1}	3.9×10^{0}
Physical Analysis												
Total Suspended Solids (mg/L)	41,900	28,800	25,500	30,600	49,100	41,600	50,300	22,000	765	50,700	22,400	23,100
Volatile Suspended Solids (mg/L)	16,800	20,000	13,700	17,000	30,300	27,400	24,900	12,900	550	30,500	16,000	15,300
Sample condition:												
pH	6.8	6.5	7.0	7.6	7.0	6.5	--	7.0	7.0	6.9	6.9	7.1
Temperature	12°C	4°C	3°C	4°C	4°C	0°C	0°C	12°C	0°C	0°C	4°C	17°C

* Red cells.

** Solids separated from liquid portion of sample and virus eluted with tryptone phosphate broth. This eluate plated directly. Liquid fraction concentrated via bentonite procedure. Virus detected in this sample came from solids fraction only.

Concentration (X) indicated with a range of values.

TABLE 15. BACTERIAL SCREEN SUMMARY—NON-ENTEROBACTERIACEAE ORGANISMS

	Tank Truck Application Sites						Spray Gun Application Sites					
	Muncie		Longmont		Cibolo		Portland		Shreveport		St. Petersburg	
Bacterial Screen	10-7-78	2-20-79	10-16-78	11-27-78	2-5-79	3-19-79	10-30-78	12-11-78	11-13-78	1-22-79	1-8-79	3-5-79
Non-Enterobacteriaceae (cfu/gm)												
Achromobacter xylosoxidans	--	--	--	--	6.1×10^5	--	--	1.5×10^4	--	--	--	4.3×10^3
Achromobacter spp. (Vd)	--	2.1×10^6	1.3×10^4	--	2.0×10^5	--	--	5.9×10^4	--	--	4.9×10^6	3.9×10^6
Acinetobacter calcoaceticus												
var. anitratus	--	--	--	--	--	--	--	4.5×10^3	6.3×10^5	--	--	1.4×10^4
var. Iwoffi	--	--	--	--	--	--	--	4.5×10^3	--	2.0×10^6	8.9×10^5	--
Aeromonas hydrophilia	2.9×10^6	6.0×10^7	7.7×10^4	1.9×10^5	2.0×10^6	4.1×10^7	2.0×10^4	5.9×10^4	1.8×10^7	5.3×10^7	1.3×10^7	2.1×10^7
Alcaligenes spp.	7.9×10^4	1.0×10^6	1.3×10^4	--	2.0×10^5	--	--	--	--	--	4.5×10^3	--
Bordetta bronchiseptica	--	--	--	--	2.0×10^5	--	--	--	--	--	--	--
CDC Group II K-2	--	--	--	--	--	--	--	4.5×10^3	--	--	--	--
CDC Group IV C-2	--	--	--	--	--	--	--	1.5×10^4	--	--	--	--
CDC Group IV E	--	--	--	--	--	2.4×10^6	--	--	--	--	4.5×10^3	4.3×10^3
CDC Group V E-1	7.9×10^4	--	--	1.1×10^4	2.0×10^5	8.0×10^5	--	--	--	--	--	--
CDC Group V E-2	--	2.1×10^6	--	--	--	--	--	--	--	--	--	1.3×10^7
Eikenella corrodens	--	--	--	--	--	2.4×10^6	--	--	--	--	1.1×10^6	--
Flavobacterium odoratum	1.6×10^5	--	3.8×10^4	--	--	--	--	4.5×10^3	6.3×10^5	--	2.2×10^5	2.9×10^6
Pasteurella aerogenes	--	--	--	--	--	--	--	--	--	--	--	4.3×10^3
Pasteurella multocida	--	--	--	--	--	--	--	--	--	--	--	2.8×10^6
Pseudomonas aeruginosa	7.9×10^4	--	1.3×10^4	8.4×10^4	--	--	--	--	--	--	8.9×10^5	1.3×10^6
Pseudomonas cepacia	5.5×10^5	--	2.6×10^4	--	--	5.6×10^5	--	4.5×10^4	--	9.8×10^5	3.1×10^6	1.4×10^6
Pseudomonas fluorescens	3.1×10^5	1.0×10^6	3.8×10^4	4.2×10^4	--	1.2×10^6	2.0×10^4	1.8×10^4	3.1×10^6	9.8×10^6	1.1×10^6	4.3×10^5
Pseudomonas maltophilia	--	--	1.3×10^4	--	--	--	2.0×10^6	--	--	9.8×10^5	--	1.4×10^6
Pseudomonas paucimobilis	--	--	--	--	--	--	--	--	--	9.8×10^5	4.5×10^5	--
Pseudomonas putida	6.2×10^5	4.2×10^6	1.4×10^5	5.2×10^4	4.7×10^6	1.3×10^7	4.0×10^4	9.0×10^4	4.4×10^6	1.3×10^7	3.1×10^6	1.1×10^7
Pseudomonas putrefaciens	--	--	--	--	--	--	--	4.5×10^3	--	9.8×10^5	--	--
Pseudomonas spp. (other)	--	1.0×10^6	--	--	--	--	--	--	--	--	8.9×10^5	1.3×10^7
Pseudomonas stutzeri	3.1×10^5	--	--	--	--	--	--	1.5×10^4	6.3×10^5	9.8×10^5	4.5×10^5	--
Vibrio alginolyticus	--	1.0×10^6	--	--	2.0×10^5	--	--	--	--	9.8×10^5	2.2×10^5	--
Vibrio parahemolyticus	--	--	--	--	4.0×10^5	--	--	--	--	--	--	--

TABLE 16. BACTERIAL SCREEN SUMMARY—ENTEROBACTERIACEAE ORGANISMS

	Tank Truck Application Sites						Spray Gun Application Sites					
	Muncie		Longmont		Cibolo		Portland		Shreveport		St. Petersburg	
Bacterial Screen	10-7-78	2-20-79	10-16-78	11-27-78	2-5-79	3-19-79	10-30-78	12-11-78	11-13-78	1-22-79	1-8-79	3-5-79
Enterobacteriaceae (cfu/gm)												
Citrobacter diversus-Levinae	--	--	--	--	1.0×10^6	--	--	--	--	--	--	--
Citrobacter freundii	7.1×10^5	2.1×10^6	5.2×10^4	9.7×10^4	6.1×10^5	3.2×10^6	2.8×10^5	4.5×10^3	1.9×10^6	5.9×10^6	4.5×10^5	2.9×10^6
Enterobacter aerogenes	--	--	--	2.2×10^4	--	--	4.0×10^4	1.4×10^4	8.8×10^6	2.0×10^6	2.2×10^5	4.3×10^5
Enterobacter agglomerans	2.4×10^5	1.6×10^7	5.1×10^4	6.3×10^4	--	2.4×10^6	1.0×10^5	8.8×10^4	3.8×10^6	4.9×10^6	8.9×10^5	4.3×10^6
Enterobacter cloacae	1.6×10^5	--	1.5×10^5	1.3×10^5	1.0×10^6	4.1×10^6	5.0×10^5	5.9×10^4	8.1×10^6	1.6×10^7	1.3×10^6	5.7×10^5
Enterobacter sakazakii	--	--	--	1.2×10^4	2.0×10^5	--	6.0×10^4	4.5×10^3	--	9.8×10^5	--	--
Escherichia coli	1.7×10^6	1.8×10^7	1.9×10^5	7.7×10^4	2.0×10^5	7.3×10^6	2.6×10^4	6.0×10^4	1.4×10^7	8.8×10^6	2.6×10^6	4.3×10^6
Hafnia alvei	--	--	--	--	8.0×10^6	--	--	--	--	--	8.9×10^5	--
Klebsiella oxytoca	6.2×10^5	6.3×10^6	2.8×10^5	2.8×10^5	6.1×10^5	2.4×10^7	2.8×10^5	2.4×10^5	--	9.8×10^5	1.4×10^6	1.4×10^6
Klebsiella ozaenae	7.9×10^4	2.1×10^6	2.6×10^4	8.4×10^4	2.7×10^6	--	6.6×10^3	--	--	9.8×10^5	8.9×10^5	1.4×10^6
Klebsiella pneumoniae	1.6×10^5	--	5.0×10^4	4.2×10^4	6.1×10^5	1.2×10^7	1.0×10^5	2.0×10^5	1.9×10^6	2.0×10^6	4.2×10^6	5.7×10^6
Proteus mirabilis	--	--	--	1.1×10^4	--	--	--	--	--	--	--	--
Proteus morganii	--	--	--	--	2.0×10^5	--	--	--	--	--	--	--
Serratia liquefaciens	7.9×10^4	--	1.3×10^4	4.2×10^4	--	1.2×10^6	8.0×10^4	--	3.1×10^6	3.9×10^6	1.3×10^6	--
Serratia marcescens	--	--	--	--	--	--	--	--	--	9.8×10^5	--	--
Serratia rubidaea	--	--	2.6×10^4	--	--	--	6.6×10^3	4.5×10^3	6.3×10^5	2.0×10^6	--	8.7×10^5
Yersinia enterocolitica	--	--	--	--	2.0×10^5	1.2×10^6	--	--	6.3×10^5	--	4.5×10^5	--
Yersinia ruckeri	--	--	--	--	1.0×10^6	--	--	--	--	--	--	--

TABLE 17. DATA SUMMARY FOR ROUTINE MICROBIOLOGICAL AND PHSYCIAL ANALYSES IN SLUDGE

Analysis	MAXIMUM	MINIMUM	MEAN*	ST. DEV.*	CONFIDENCE LIMITS UPPER	CONFIDENCE LIMITS LOWER
Bacteria (cfu/gm)						
Standard Plate Count	7.3×10^{10}	4.8×10^{6}	3.0×10^{8}	2.75	1.9×10^{9}	4.7×10^{7}
Total Coliform	6.1×10^{7}	2.1×10^{5}	4.9×10^{6}	1.95	1.9×10^{7}	1.4×10^{6}
Fecal Coliform	7.8×10^{6}	1.2×10^{4}	6.2×10^{5}	2.15	2.6×10^{6}	1.5×10^{5}
Fecal streptococci	1.1×10^{7}	6.8×10^{4}	4.5×10^{5}	1.78	1.5×10^{6}	1.3×10^{5}
Fluorescent pseudomonads	3.7×10^{5}	$<6.6 \times 10^{2}$	1.4×10^{4}	2.64	8.4×10^{4}	2.4×10^{3}
Clostridium perfringens						
- vegetative (MPN/gm)	2.0×10^{8}	2.1×10^{6}	1.6×10^{7}	1.28	3.8×10^{7}	6.9×10^{6}
- sporulated (MPN/gm)	7.7×10^{7}	5.0×10^{5}	4.7×10^{6}	1.46	1.3×10^{7}	1.8×10^{6}
Mycobacteria sp.	2.4×10^{9}	$<1.1 \times 10^{6}$	1.9×10^{7}	2.89	1.3×10^{8}	2.7×10^{6}
Klebsiella sp.	2.9×10^{7}	1.1×10^{5}	1.7×10^{6}	1.91	6.1×10^{6}	4.7×10^{5}
Viruses (pfu/gm)						
Bacteriophage	2.3×10^{5}	1.4×10^{3}	4.7×10^{4}	1.52	1.3×10^{5}	1.7×10^{4}
Enteroviruses HeLa	1.7×10^{1}	3.0×10^{-1}	1.6	2.19	3.8	6.7×10^{-1}
Physical (mg/L)						
TSS	50,700	22,000	35,100	11,800	43,000	27,200
USS	30,500	12,900	21,000	6,890	25,600	16,400

*Geometric mean and standard deviation for microbiological analyses, arthmetic for physical analyses

TABLE 18. DATA SUMMARY FOR NON-ENTEROBACTERIA IN SLUDGE
(cfu/gm)

	Aeromonas hydrophilia	Pseudomonas cepacia	Pseudomonas fluorescens	Pseudomonas putida
Maximum	6.0×10^7	5.6×10^6	9.8×10^6	1.3×10^7
Minimum	2.0×10^4	2.6×10^4	1.8×10^4	4.0×10^4
Geometric Mean	2.5×10^6	5.5×10^5	3.1×10^5	1.2×10^6
Geometric St. Dev.	2.93	2.05	2.15	2.25
Confidence Limits				
Upper	1.6×10^7	3.7×10^6	1.3×10^6	5.0×10^6
Lower	3.9×10^5	8.2×10^4	7.4×10^4	2.8×10^5

The Enterobacteriaceae organisms found in samples from all sites (although not necessarily in all samples) were: Citrobacter freundii, Enterobacter agglomerans, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella ozaenae, Klebsiella pneumoniae, and Serratia liquefaciens. In contrast, Citrobacter direreus-Levine, Hafnia alvei, Proteus mirabilis and Yersinia ruckeri were each detected in only one of the twelve samples. The results for those organisms detected at all sites are summarized in Table 19. Geometric means for these organisms were calculated and ranged from 2.7 x 10^5 cfu/g for Klebsiella ozaenae to 9.5 x 10^5 cfu/g for Klebsiella oxytoca, and the minima and maxima similarly varied little from one organism to another. The geometric standard deviation was used to calculate a confidence interval on the mean in the natural logarithm-transformed scale, and the results exponentiated to give the limits shown in the table. As with the trace metal data, these limits may be used to estimate the expected concentrations of these organisms in liquid sludge, under the assumption that the plants studied are representative.

MICROBIOLOGICAL AEROSOL STUDY

Tank Truck Sites

Microbiological Results--

Five microbiological aerosol runs were made at Longmont and eight at Cibolo. Microbiological constituents sought at the two sites were bacteriophage, standard plate count, total coliform, fecal coliform, and fecal streptococci. The runs were made under conditions expected to result in high levels of the organisms downwind of the field if they are aerosolized. The meteorological conditions during the aerosol runs are shown in Table 20. A sludge sample was taken with each aerosol run and analyzed for the microbiological and physical characteristics. Enteroviruses and total and volatile suspended solids were analyzed for in the sludge in addition to the organisms sought in the aerosol samples. These analyses are reported in Table 21.

The microorganisms reported in the aerosol samples were converted to either pfu or cfu, as appropriate, per cubic meter of air, and the results of the analyses are shown in Tables 22 to 26. Also shown for comparison purposes are the counts per g of TSS determined for the sludge sample and the geometric mean result for those data sets for which a significant portion of the total analyses were above the detection limit.

Data Analysis--

The results were used to evaluate the effect on the microorganisms of the spraying operation. The microbiological disperson model used to evaluate these results may be represented as

$$C_d = Q \cdot D_d \cdot e^{\lambda a d} + B \quad , \qquad (1)$$

TABLE 19. DATA SYMMARY FOR ENTEROBACTERIACEAE IN SLUDGE

	Organism							
	Citrobacter freundii	*Enterobacter agglomerans*	*Enterobacter cloacae*	*Escherichia coli*	*Klebsiella oxytoca*	*Klebsiella ozonae*	*Klebsiella pneumoniae*	*Serratia liquefaciens*
Maximum	5.9×10^6	1.6×10^7	1.6×10^7	1.8×10^7	2.4×10^7	2.7×10^6	1.2×10^7	3.9×10^6
Minimum	4.5×10^3	5.1×10^4	5.9×10^4	2.6×10^4	2.4×10^3	6.6×10^3	4.2×10^4	1.3×10^4
Geometric Mean	5.0×10^5	7.0×10^5	7.8×10^5	8.2×10^5	9.5×10^5	2.7×10^5	6.1×10^5	3.0×10^5
Geometric Sta. Dev.	2.07	2.08	1.91	2.35	1.45	2.16	2.00	2.18
Confidence Limits								
Upper	1.8×10^6	2.8×10^6	2.8×10^6	3.6×10^6	2.5×10^6	1.4×10^6	2.4×10^6	1.8×10^6
Lower	1.4×10^5	1.7×10^5	2.2×10^5	1.9×10^5	3.6×10^5	5.1×10^4	1.6×10^5	4.9×10^4

TABLE 20. METEOROLOGICAL DATA SUMMARY-TANK TRUCK SITES

Site	Run	Date	Run* Time, min	Met Tower	Air Temperature, °C Wet Bulb	Air Temperature, °C Dry Bulb	Relative Humidity, %	Cloud Cover, eighths	Cloud Type	Black Tower Wind Direction, deg	Black Tower Wind Speed, m/sec	White Tower Wind Direction, deg	White Tower Wind Speed m/sec	Solar Radiation
Longmont	1	8-7-79	13:30	32	16	29	26	0	N/A	20-180	2.6	0-190	2.2	1.01
	2	8-8-79	15:35	30	17	27	41	0	N/A	300-160	2.4	90-200	2.4	0.70
	3	8-13-79	17:08	18	14	18	55	8	---†	180-010	1.5	180-010	1.6	0.15
	4	8-21-79	14:51	23	14	24	36	2	---	010-140	1.8	10-180	1.8	0.94
	5	8-22-79	17:07	23	14	22	41	2	---	020-150	1.9	10-210	2.2	0.66
Cibolo	1	10-1-79	18:28	--	21	23	--	6	L6	160-250	2.9	150-2[illegible]	2.8	0.44
	2	10-2-79	19:28	--	18	27	--	0	N/A	340-050	3.3	310-0[illegible]	---	0.73
	3	10-24-79	17:30	--	11	18	--	0	N/A	000-140	1.6	40-30[illegible]	1.8	0.44
	4	10-25-79	18:00	--	15	22	--	0	N/A	90-250	2.9	90-200	2.6	0.67
	5	10-30-79	12:00	--	22	26	71	8	L5, L7	130-190	6.2	110-170	---	0.03
	6	11-1-79	18:00	--	9	16	40	1	H4	060-70	5.8	---	---	0.58
	7	11-13-79	18:00	--	9	14	51	0	N/A	350-160	3.6	350-060	3.4	0.70
	8	11-14-79	18:00	--	7	13	34	0	N/A	20-180	2.7	060-140	3.0	0.65

* From downwind 2 position
† Not available.

TABLE 21. ENTEROVIRUSES AND PHYSICAL ANALYSES - TANK TRUCK SITES

Site	Run	Enteroviruses (pfu/gm TSS) HeLa	Enteroviruses (pfu/gm TSS) Hep-2	TSS (mg/L)	VSS (mg/L)	Sample Condition pH	Sample Condition Temperature (°C)
Longmont	1	1.7	-*	25,000	13,200	7.5	12
	2	1.7	-	22,000	11,900	7.5	8
	3	0.33	-	35,800	18,600	7.5	6
	4	0.26	-	51,600	24,400	7.5	11
	5	0.35	-	52,600	23,700	7.4	6
Cibolo	1	1.9	0.93	49,300	26,800	6.9	30
	2	1.2	2.0	49,500	27,100	6.6	26
	3	0.35	0.12	55,000	19,700	6.6	27
	4	2.1	<0.1	50,000	19,700	6.8	24
	5	1.6	0.30	49,900	26,400	6.9	-
	6	1.6	0.30	49,900	26,400	6.9	24
	7	6.0	1.5	45,500	24,900	6.6	19
	8	0.50	0.40	54,600	29,000	6.8	17

*Not reported

TABLE 22. CONCENTRATION OF BACTERIOPHAGE IN AEROSOL SAMPLES TANK TRUCK SITES (pfu/m³)

Site	Run	Sludge Sample (pfu/g TSS)	Sampler Position		
			Upwind	Downwind 1	Downwind 2
Longmont	1	1.6×10^5	<0.7	4.2	<0.4
	2	1.4×10^5	<0.9	<0.9	CS
	3	1.1×10^5	<0.4	<0.4	<0.4
	4	6.8×10^4	<0.7	0.4	0.4
	5	5.7×10^4	<1.6	<0.4	<0.4
$\bar{X}_G$		9.9×10^4	—	—	—
Cibolo	1	2.2×10^5	<0.4	2.4	2.1
	2	1.3×10^5	<0.4	<0.4	<0.4
	3	1.0×10^5	<0.4	<0.4	0.7
	4	1.7×10^5	<0.4	<0.4	<0.4
	5	1.3×10^5	<0.4	1.3	<0.4
	6	1.0×10^5	<0.4	<0.4	<0.4
	7	2.4×10^5	<0.4	0.4	0.4
	8	1.4×10^5	<0.4	0.4	0.4
$\bar{X}_G$		1.5×10^5	—	—	—

TABLE 23. CONCENTRATION OF STANDARD PLATE COUNT IN AEROSOL SAMPLES TANK TRUCK SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	Downwind 1	Downwind 2
Longmont	1	1.6×10^8	6.0×10^3	6.4×10^4	3.1×10^5
	2	1.3×10^8	6.0×10^4	2.2×10^5	3.3×10^6
	3	8.3×10^7	4.2×10^4	9.6×10^6	2.9×10^8
	4	5.8×10^7	7.8×10^1	1.5×10^5	9.1×10^7
	5	7.2×10^7	CS*	1.9×10^4	5.8×10^5
	$\bar{X}_G$	9.4×10^7	6.0×10^3	2.1×10^5	6.9×10^6
Cibolo	1	1.7×10^9	1.3×10^4	1.2×10^5	1.9×10^5
	2	2.4×10^8	2.4×10^4	2.2×10^6	1.3×10^5
	3	1.0×10^8	2.2×10^4	3.6×10^5	1.5×10^{10}
	4	1.7×10^8	1.1×10^6	2.2×10^6	1.1×10^5
	5	1.7×10^8	1.8×10^6	1.4×10^5	3.1×10^7
	6	1.7×10^8	3.6×10^5	1.1×10^7	2.7×10^5
	7	6.9×10^8	3.6	4.7×10^6	2.2×10^5
	8	2.0×10^8	2.7×10^5	5.3×10^5	5.6×10^6
	$\bar{X}_G$	2.7×10^8	3.8×10^4	9.8×10^5	1.2×10^6

*CS—contaminated sample

TABLE 24. CONCENTRATION OF TOTAL COLIFORM IN AEROSOL SAMPLES TANK TRUCK SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	Downwind 1	Downwind 2
Longmont	1	3.0×10^6	1.5	5.8×10^3	7.1×10^1
	2	3.5×10^6	7.1×10^1	7.1×10^4	CS*
	3	1.6×10^6	3.8	1.8×10^3	4.2×10^1
	4	1.2×10^6	5.3×10^1	1.2×10^1	<0.60
	5	3.8×10^5	—	8.2×10^2	<0.42
	$\bar{X}_G$	1.5×10^6	3.8	6.0×10^2	2.7
Cibolo	1	4.3×10^5	0.44	2.4	2.1×10^1
	2	3.2×10^6	6.4	1.1×10^2	CS
	3	5.3×10^5	0.47	1.9×10^3	7.1
	4	5.8×10^5	0.82	3.1	1.4×10^1
	5	1.5×10^6	0.62	<0.62	1.2×10^1
	6	6.0×10^5	0.40	$>1.3 \times 10^3$	5.3
	7	1.1×10^7	<0.40	$>2.2 \times 10^3$	4.0
	8	2.0×10^6	2.4×10^1	<0.40	1.2×10^1
	$\bar{X}_G$	1.3×10^6	1.7	2.0×10^1	9.6

*CS—contaminated sample

TABLE 25. CONCENTRATION OF FECAL COLIFORM IN AEROSOL SAMPLES TANK TRUCK SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/gm TSS)	Sampler Position Upwind	Downwind 1	Downwind 2
Longmont	1	2.1×10^6	$<2.3 \times 10^{-1}$	8.1	7.4
	2	1.5×10^6	9.3×10^{-1}	$<2.1 \times 10^{-1}$	CS
	3	4.2×10^5	$<2.8 \times 10^{-1}$	$<1.9 \times 10^{-1}$	2.3×10^1
	4	1.5×10^5	$<2.3 \times 10^{-1}$	$<2.3 \times 10^{-1}$	2.7×10^{-1}
	5	1.3×10^5	$<5.5 \times 10^{-1}$	$<1.9 \times 10^{-1}$	1.9×10^{-1}
	$\bar{X}_G$	4.8×10^5	—	—	1.05
Cibolo	1	8.6×10^4	$<2.0 \times 10^{-1}$	1.8×10^{-1}	5.5×10^{-1}
	2	4.0×10^5	$<1.7 \times 10^{-1}$	1.7×10^{-1}	1.7×10^{-1}
	3	9.8×10^4	2.1×10^{-1}	$<2.1 \times 10^{-1}$	1.9×10^{-1}
	4	2.2×10^5	1.7	2.6	$<1.9 \times 10^{-1}$
	5	1.9×10^5	$<2.8 \times 10^{-1}$	12.8×10^{-1}	4.2
	6	8.8×10^4	$<1.8 \times 10^{-1}$	5.6	$<1.8 \times 10^{-1}$
	7	8.9×10^5	$<1.8 \times 10^{-1}$	$>1 \times 10^3$	8.9×10^{-1}
	8	1.4×10^5	8.9×10^2	1.8×10^{-1}	5.6
	$\bar{X}_G$	12.0×10^5	—	5.3×10^{-1}	5.5×10^{-1}

TABLE 26. CONCENTRATION OF FECAL STREPTOCOCCI IN AEROSOL SAMPLES TANK TRUCK SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	Downwind 1	Downwind 2
Longmont	1	8.0×10^5	<0.51	2.2×10^1	4.0×10^1
	2	3.1×10^5	2.7	1.5	7.1×10^1
	3	2.3×10^5	<0.62	3.3	6.9
	4	1.2×10^5	0.51	1.0×10^1	9.6
	5	2.3×10^5	2.4	1.3	6.0
	$\bar{X}_G$	2.8×10^5	0.58	4.2	1.6×10^1
Cibolo	1	2.0×10^4	4.9	3.8	2.0
	2	7.8×10^4	3.1	3.6	1.2
	3	1.8×10^4	2.4	2.2	0.22
	4	4.2×10^4	2.0	5.1	3.1
	5	2.2×10^4	2.2×10^1	1.4×10^1	3.1×10^1
	6	1.7×10^5	<0.40	1.2	<0.40
	7	2.7×10^5	0.40	3.1×10^1	7.3
	8	5.1×10^4	1.2	1.2	<0.40
	$\bar{X}_G$	5.3×10^4	1.7	2.7	1.2

where C_d - downwind concentration
Q - source strength
D_d - diffusion factor
λ - organism decay rate
a_d - aerosol age
and B - background concentration.

The source strength, Q, is determined from the concentration of the microorganisms in the sludge sample (W), the rate of application (F), the aerosolization efficiency (E), and the effect of aerosolization on the organisms (I). Assigning terms for each of these parameters gives

$$Q = W \cdot F \cdot E \cdot I \quad ,$$

and substituting into equation (1) gives

$$C_d = W \cdot F \cdot E \cdot I \cdot D_d \cdot e^{\lambda a_d} + B \qquad (2)$$

or

$$(C_d - B)/W \cdot F \cdot D_d = E \cdot I \cdot e^{\lambda a_d} \quad . \qquad (3)$$

For the tank truck sites, C and B were measured in the collecting media, W was determined from the sludge sample, F was the pumping rate, and D_d is estimated from the physical layout of the sampling site. For these sites, the sampling stations were established as close as possible to the spray area, and the die-off (λ) was assumed to be zero, making that term go to unity. There was no way to determine aerosolization efficiency, and the individual results were used to calculate E·I estimates for each microbiological constituent.

The Gaussian form of the diffusion factor is calculated as (13)

$$D_d(y,z) = \frac{1}{2\pi u \sigma_y \sigma_z} e^{-\frac{1}{2}\left(\frac{y}{\sigma_y}\right)^2} \left[e^{-\frac{1}{2}\left(\frac{z-H}{\sigma_z}\right)^2} + e^{-\frac{1}{2}\left(\frac{z+H}{\sigma_z}\right)^2} \right]$$

where y - distance in cross-wind direction, (m)
z - distance in vertical direction, (m)
u - mean wind speed, (m/sec)
σ_y - standard deviation of plume concentration distribution in the crosswind direction, (m)
σ_z - standard deviation of plume concentration distribution in the vertical direction, (m)
H - height of plume center-line, (m).

For these areosol runs, the samplers were considered to be downwind of the spray site while the truck was parallel to the sampling sites along the path of the prevailing wind. The average width of the path for the passes on any run was used to represent both y and $4\sigma_y$, and the σ_y determined from this. The vertical plume height standard deviation, σ_z, was estimated by assuming all aerosol to be generated at the ground level, reaching a height of 2 m at the sampling point. The pattern of distribution was assumed to be a truncated normal distribution, with the ground serving as a truncating barrier. The center of the distribution was assumed to be at 0.5 m above ground, so the remaining 1.5 m represented the $2\sigma_z$ limit for the normal portion of the distribution. Thus, a σ_z of $1.5/2 = 0.75$ m was used on all aerosol runs. The diffusion factors were calculated according to these assumptions and substituted into equation (3) to obtain E·I estimates. If background levels were below detection limits, one-fourth of the detection limit was used instead.

Since the E factor would be the same for all microbiological components, the E·I can be ratioed against a common indicator to provide relative impact factors. Standard plate count was used as the denominator since it was the most prevalent of the E·I estimates, and ratios were calculated against fecal coliform when standard plate count was not available. Table 27 gives the relative impact factors and shows summary statistics that can be used to compare the impact of this type of application to that from a wastewater site. These comparisons are made in Section 7, Discussion.

Spray Sites

There were seven aerosol runs at the Portland site and eight at the St. Petersburg site. The microbiological constituents sought included those sought at the truck sites, along with mycobacteria at both sites and Salmonella at St. Petersburg. The results of the screening analyses on the sludge at St. Petersburg indicated unusually high levels of Salmonella, which warranted its selection as a study organism for that site. Runs were made under conditions expected to yield high downwind levels of any aerosolized organisms. The meteorological conditions for these two sites are given in Table 28.

The concentrations of microorganisms were reported by the analytical laboratory in relation to the milliliters of collecting medium. These were converted to counts per cubic meter of air sampled, as with the truck sites, and are shown in Tables 29 to 35.

At the spray sites, there were also high-volume particulate air samplers operating with each pair of samplers that were used to indicate the amount of particulate matter collected during each run. The microbiological concentrations were also converted to counts per milligram of particulate matter under the assumption that they would be associated with the particulate phase of the sludge. These results are shown in Tables 36 to 42.

TABLE 27. RELATIVE IMPACT FACTORS FOR TANK TRUCK SITES (STANDARD PLATE COUNT=100)

Site	Run	Position	Bacteriophage	Total Coliform	Fecal Coliform	Fecal Streptococci
Longmont	1	DW1	0.02	0.01	0.004	0.03
		DW2	--	0.05	0.02	0.11
	2	DW1	--	0.69	--	--
		DW2	--	--	--	0.21
	3	DW1	--	0.04	--	0.0001
		DW2	7×10^{-6}	--	2×10^{-5}	8×10^{-6}
	4	DW1	0.002	--	--	0.03
		DW2	3×10^{-6}	--	2×10^{-6}	5×10^{-5}
	5	DW1	--	0.82	--	--
		DW2	--	1×10^{-4}	--	0.003
Cibolo	1	DW1	0.17	0.07	0.05	--
		DW2	0.09	0.44	0.12	
	2	DW1	--	1×10^{-4}	7×10^{-5}	6×10^{-4}
		DW2	--	--	0.001	--
	3	DW1	--	1.12	--	--
		DW2*	0.14	0.001	1.00	--
	4	DW1	--	0.02	0.001	0.02
		DW2	--	--	--	--
	5	DW1*	--	3.6	1.00	--
		DW2	--	1×10^{-6}	9×10^{-6}	7×10^{-5}
	6	DW1	--	0.08	0.002	0.0001
		DW2	--	--	--	--
	7	DW1	0.0002	0.06	0.08	0.02
		DW2	0.004	0.001	0.007	0.08
	8	DW1	0.002	--	--	--
		DW2	--	--	--	--

* Relative to fecal coliform.

TABLE 28. METEOROLOGICAL DATA SUMMARY-SPRAY SITES

Site	Run	Date	Run Time,* min	Air Temperature, °C Wet Bulb	Air Temperature, °C Dry Bulb	Relative Humidity, %	Cloud Cover, eighths	Cloud Type	Black Tower Wind Direction, deg	Black Tower Wind Speed, m/sec	White Tower Wind Direction, deg	White Tower Wind Speed, m/sec	Solar Radiation
Portland	1	7-10-79	69.4	15	21	46	8	L4, L8	120-210	3.4	---	---	0.60
	2	7-11-79	58	14	19	14	8	L4	100-235	4.6	120-210	---	0.39
	3	7-11-79	77.3	16	23	46	8	L4	140-240	4.8	150-250	---	0.55
	4	7-13-79	79.2	14	19	56	0	N/A	350-040	2.1	270-030	1.7	0.99
	5	7-15-79	98.2	18	26	48	0	N/A	300-005	3.8	300-010	3.2	1.09
	6	7-16-79	81.4	20	33	25	0	N/A	305-360	2.8	285-360	2.8	0.87
	7	7-17-79	99	20	30	41	0	N/A	300-010	2.6	---	---	1.04
St. Petersburg	1	12-3-79	60	14	19	61	8	L2, L4	045-090	4.8	015-060	4.2	0.35
	2	12-4-79	60	14	17	74	3	H6, M9	010-090	3.7	000-060	3.3	0.54
	3	12-4-79	60	16	21	64	5	H6, M9	000-070	3.2	340-060	3.4	0.51
	4	12-5-79	60	18	22	68	8	M3, L8	090-150	1.2	120-140	1.0	0.39
	5	12-6-79	60	22	25	79	8	L4, M7	140-200	9.2	140-210	8.0	0.30
	6	12-10-79	60	18	25	48	3	L4,H1,H2	000-080	2.8	---	---	0.21
	7	12-12-79	60	21	27	58	5	H6, L2	040-120	3.6	040-130	3.8	0.59
	8	12-12-79	60	22	29	52	1	H8, L1	030-120	3.0	060-130	3.4	0.54
	Virus	12-11-79	1,470	20	26	60	8	L2,L4,M6	060-090	2.7	N/A	N/A	0.32

* From 50 m downwind position.

TABLE 29. CONCENTRATION OF BACTERIOPHAGE IN AEROSOL SAMPLES SPRAY SITES (pfu/m³)

Site	Run	Sludge Sample (pfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	1.9×10^4	<0.27	<0.27	0.27	<0.27
	2	1.4×10^4	$<2.0 \times 10^3$	$<3.1 \times 10^1$	0.62	0.31
	3	1.6×10^4	<0.24	0.22	0.22	0.24
	4	9.9×10^3	<0.22	0.44	0.22	0.22
	5	2.2×10^4	<0.20	0.53	0.18	0.18
	6	1.8×10^4	<0.24	1.1	1.1	0.22
	7	7.7×10^3	0.36 /0.49	<0.18	0.53	0.18
	$\bar{X}_G$	1.4×10^4	ND*	0.33	0.36	0.13
St. Petersburg	1	5.6×10^4	<1.6	4.4	1.8	1.2×10^2
	2	1.1×10^5	<0.22	3.6	1.6	0.22
	3	1.2×10^5	<0.22	4.2	3.6	0.67
	4	2.8×10^4	<0.22	5.1	4.2	0.89
	5	1.5×10^5	<0.22	1.8	2.1	0.67
	6	7.3×10^4	<0.22	0.67	0.89	2.7
	7	1.1×10^5	<0.22	0.89	1.2	0.67
	8	7.8×10^4	<0.44	0.22	1.8	0.44
	$\bar{X}_G$	8.1×10^4	ND	1.7	1.9	0.73

*ND—not detected

TABLE 30. CONCENTRATION OF STANDARD PLATE COUNT IN AEROSOL SAMPLES SPRAY SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	6.6×10^7	8.0×10^2	1.5×10^3	1.8×10^3	4.4×10^2
	2	6.7×10^7	1.8	4.4×10^3	5.6×10^3	—
	3	1.5×10^7	1.2×10^2	1.1×10^2	4.2×10^2	1.2×10^2
	4	1.8×10^7	1.6×10^3	1.4×10^4	1.4×10^3	CS*
	5	1.2×10^6	2.7×10^5	2.9×10^5	2.4×10^5	6.4×10^6
	6	1.0×10^8	1.3×10^5	3.8×10^3	1.1×10^5	2.9×10^6
	7	6.5×10^6	8.4×10^1 /1.2×10^4	2.7×10^3	3.8×10^3	4.0×10^3
	$\overline{X}_G$	1.9×10^7	9.6×10^2	4.2×10^3	7.1×10^3	2.1×10^4
St. Petersburg	1	7.6×10^5	6.0×10^4	4.9×10^3	1.1×10^4	2.1×10^5
	2	1.6×10^7	2.7×10^4	3.1×10^4	2.9×10^6	2.9×10^6
	3	1.5×10^9	3.1×10^5	8.2×10^4	4.4×10^4	5.3×10^5
	4	3.6×10^5	1.9×10^4	4.2×10^5	6.2×10^4	6.4×10^5
	5	1.3×10^8	1.3×10^5	3.1×10^4	3.1×10^4	5.8×10^4
	6	1.5×10^9	1.6×10^3	1.8×10^4	4.0×10^5	4.9×10^5
	7	1.6×10^9	3.8×10^5	2.9×10^4	9.6×10^3	1.5×10^4
	8	8.6×10^8	5.6×10^3	3.3×10^5	5.8×10^5	1.4×10^5
	$\overline{X}_G$	8.0×10^7	5.1×10^4	4.9×10^4	9.3×10^4	2.4×10^5

*CS—contaminated sample

TABLE 31. CONCENTRATION OF TOTAL COLIFORM IN AEROSOL SAMPLES SPRAY SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	2.9×10^6	ND*	2.7	1.9	1.7
	2	2.2×10^6	<0.27	1.1×10^1	1.0×10^1	4.7
	3	7.0×10^5	<0.20	0.38	<0.20	<0.20
	4	3.1×10^5	<0.18	0.89	1.1	0.20
	5	1.4×10^6	<0.16	1.5	0.44	0.13
	6	1.6×10^6	0.20	1.8	1.3	0.18
	7	8.9×10^4	<0.33	0.16	1.4	1.0×10^1
	$\bar{X}_G$	8.3×10^5	ND*	1.2	1.0	0.58
St. Petersburg	1	8.9×10^2	1.8	2.9×10^1	2.0×10^1	2.1×10^1
	2	4.7×10^7	<0.44	2.9×10^1	7.3×10^1	1.0×10^1
	3	2.0×10^7	<0.44	4.2×10^1	4.9×10^1	1.8×10^1
	4	6.4×10^2	2.9	1.4×10^2	3.3×10^1	2.4×10^1
	5	1.5×10^7	0.44	2.2×10^1	2.2×10^1	2.4×10^1
	6	1.4×10^7	<0.44	7.3	2.9	2.7
	7	1.8×10^7	6.2×10^1	5.6×10^1	1.6×10^1	4.9
	8	5.9×10^6	2.9	2.9	1.5×10^2	2.9×10^1
	$\bar{X}_G$	1.4×10^6	0.93	2.4×10^1	2.4×10^1	1.3×10^1

*ND—not detected

TABLE 32. CONCENTRATION OF FECAL COLIFORM IN AEROSOL SAMPLES SPRAY SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	3.1×10^5	<0.22	0.22	0.22	<0.22
	2	4.4×10^5	1.9×10^1	5.3	5.8	0.24
	3	1.2×10^5	<0.20	<0.20	<0.20	<0.20
	4	4.9×10^4	<0.18	0.18	0.38	<0.20
	5	1.8×10^5	<0.16	0.44	<0.16	<0.13
	6	3.2×10^5	<0.20	1.4	0.18	0.18
	7	1.5×10^4	<0.33	0.29	0.16	<0.16
	$\bar{X}_G$	1.8×10^5	ND*	0.40	0.22	ND
St. Petersburg	1	1.8×10^3	0.73	1.7×10^1	1.3×10^1	3.3
	2	9.2×10^6	<0.24	2.7×10^1	$<1.5 \times 10^2$	4.0
	3	1.2×10^7	<0.22	2.9×10^1	2.7×10^1	1.6×10^1
	4	6.4×10^2	1.2	2.7×10^2	1.4×10^1	9.6
	5	2.8×10^5	<0.22	6.7	1.1×10^1	1.0×10^1
	6	2.9×10^6	0.49	8.9	4.4	1.5
	7	4.1×10^6	0.73	0.96	4.0	3.8
	8	1.3×10^6	<0.24	0.73	3.8	6.2
	$\bar{X}_G$	3.9×10^5	2.0	1.0×10^1	8.7	5.4

*ND—not detected

TABLE 33. CONCENTRATION OF FECAL STREPTOCOCCI IN AEROSOL SAMPLES SPRAY SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	1.4×10^5	1.1	1.0	7.1	0.64
	2	1.1×10^5	0.16	9.1	1.4×10^1	6.0
	3	4.5×10^4	0.40	1.2	1.2	0.38
	4	2.5×10^4	0.38	1.1	0.89	1.3
	5	1.3×10^5	0.16	4.9	2.1	0.13
	6	9.1×10^4	0.78	7.6	5.3	0.18
	7	0.3×10^3	<0.32	1.2	0.58	0.29
	$\bar{X}_G$	5.7×10^4	0.31	2.5	2.5	0.76
St. Petersburg	1	4.1×10^4	0.24	1.8×10^1	1.3×10^1	8.2
	2	1.0×10^6	<0.24	2.2×10^1	1.8×10^1	9.6
	3	7.5×10^5	<0.22	4.2×10^1	5.1×10^1	2.2×10^1
	4	1.8×10^4	1.9	8.2×10^1	7.1×10^1	4.0×10^1
	5	9.7×10^4	3.8	3.8×10^1	1.4×10^1	2.7×10^1
	6	7.7×10^5	1.7	3.6×10^1	1.9×10^1	1.0×10^1
	7	8.2×10^5	2.7	1.6×10^1	1.9×10^1	1.2×10^1
	8	5.0×10^5	2.9×10^5	2.2×10^1	8.2×10^1	2.7×10^1
	$\bar{X}_G$	2.5×10^5	0.60	3.0×10^1	2.8×10^1	1.7×10^1

TABLE 34. CONCENTRATION OF MYCOBACTERIUM SPP. IN AEROSOL SAMPLES SPRAY SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	8.7×10^5	0.13	0.27	0.78	ND*
	2	1.6×10^6	ND	0.47	0.31	0.16
	3	1.8×10^6	2.7	1.8	1.2	2.7
	4	2.8×10^7	<0.11	1.5	3.3	2.1
	5	6.0×10^6	0.20	0.18	1.4	<0.09
	6	1.3×10^6	<0.11	0.22	0.11	<1.1
	7	4.3×10^6	0.20/0.09	0.44	<0.09	0.09
	$\bar{X}_G$	4.2×10^6	0.13	0.53	0.44	0.18
St. Petersburg	1	3.8×10^7	0.29	6.2	6.0	8.2
	2	6.4×10^7	<0.16	7.3	5.8	1.9
	3	6.4×10^7	0.22	5.6	7.3	6.9
	4	5.9×10^7	CS	CS	CS	CS †
	5	1.5×10^6	<0.16	1.2×10^1	5.8	<0.16
	6	6.1×10^7	0.16	5.8	8.2	1.5×10^1
	7	$>2.0 \times 10^5$ (CS)	0.22	8.9	1.0×10^1	1.6×10^1
	8	$>1.5 \times 10^5$ (CS)	0.89	3.8	5.3	4.7
	$\bar{X}_G$	3.1×10^7	0.07	6.7	6.8	2.5

*ND—not detected

† CS—contaminated sample

TABLE 35. CONCENTRATION OF SALMONELLA IN AEROSOL SAMPLES SPRAY SITES (cfu/m³)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
St. Petersburg	1	<2.7	<0.44	<0.44	<0.44	<0.67
	2	1.5×10^3	<0.22	<0.44	0.44	<0.44
	3	<7.7	IS*	<0.44	<0.44	<0.44
	4	$<2.1 \times 10^1$	IS	<0.44	<0.44	<0.44
	5	<1.0	<0.44	<0.44	<0.44	3.8
	6	5.5	<4.4	<4.4	<0.44	<0.44
	7	<5.9	<0.44	<0.44	<0.44	<0.44
	8	4.5	<0.44	<0.44	<0.44	<0.44
	$\bar{X}_G$	—	—	—	—	—

*IS—insufficient sample

TABLE 36. CONCENTRATION OF BACTERIOPHAGE IN AEROSOL SAMPLES SPRAY SITES (pfu/mg particulate)

Site	Run	Sludge Sample (pfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	1.9×10^4	<1.4	<1.4	1.4	<1.4
	2	1.4×10^4	1.0×10^3	2.2×10^2	4.7	—
	3	1.5×10^4	<4.4	2.7	2.7	2.4
	4	9.9×10^3	<6.9	5.1	2.7	<1.6
	5	2.2×10^4	<4.0	3.3	1.1	1.2
	6	1.8×10^4	<3.8	6.4	8.0	<1.5
	7	7.7×10^3	4.9/6.9	<1.1	3.3	0.89
St. Petersburg	1	5.6×10^4	—	1.8×10^1	7.1	1.4×10^1
	2	1.1×10^5	2.9	2.9×10^1	1.2×10^1	—
	3	1.2×10^4	$<2.9 \times 10^1$	3.3×10^1	2.9×10^1	—
	4	2.8×10^4	1.0×10^1	2.2×10^1	1.9×10^1	3.3
	5	1.5×10^5	—	0.40	0.44	0.18
	6	7.3×10^4	—	1.1×10^1	1.6×10^2	7.3
	7	1.1×10^5	—	6.4	8.4	5.3
	8	7.8×10^4	<6.4	5.3	3.3×10^1	1.2

TABLE 37. CONCENTRATION OF STANDARD PLATE COUNT IN AEROSOL SAMPLES SPRAY SITES (cfu/mg particulate)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	6.6×10^{7}	9.1×10^{3}	7.8×10^{3}	9.6×10^{3}	4.0×10^{3}
	2	6.7×10^{7}	8.9×10^{3}	3.3×10^{4}	4.2×10^{4}	—
	3	1.5×10^{7}	2.2×10^{3}	1.3×10^{3}	5.1×10^{3}	1.2×10^{4}
	4	1.8×10^{7}	4.9×10^{4}	1.5×10^{5}	1.5×10^{5}	CS*
	5	1.2×10^{6}	5.6×10^{6}	6.2×10^{6}	1.7×10^{6}	1.5×10^{6}
	6	1.0×10^{8}	2.0×10^{6}	2.2×10^{4}	8.0×10^{5}	2.0×10^{7}
	7	6.5×10^{6}	1.2×10^{3}/ 1.7×10^{5}	1.5×10^{5}	3.6×10^{4}	2.0×10^{4}
St. Petersburg	1	7.6×10^{5}	—	2.0×10^{4}	4.4×10^{4}	2.4×10^{6}
	2	1.6×10^{7}	2.7×10^{6}	2.4×10^{5}	2.2×10^{7}	—
	3	1.5×10^{9}	3.1×10^{7}	6.4×10^{5}	3.6×10^{5}	—
	4	3.6×10^{5}	6.9×10^{5}	1.9×10^{6}	2.9×10^{5}	2.4×10^{6}
	5	2.3×10^{8}	—	6.9×10^{4}	6.9×10^{4}	1.6×10^{4}
	6	1.5×10^{9}	—	3.3×10^{5}	7.3×10^{6}	1.3×10^{7}
	7	1.6×10^{9}	—	2.1×10^{5}	6.9×10^{4}	1.3×10^{5}
	8	8.6×10^{8}	9.6×10^{4}	6.0×10^{6}	1.1×10^{7}	4.4×10^{5}

*CS—contaminated sample

TABLE 38. CONCENTRATION OF TOTAL COLIFORM IN AEROSOL SAMPLES SPRAY SITES (cfu/mg particulate)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position			
			Upwind	50R	50L	100
Portland	1	2.9×10^6	ND*	1.5×10^1	1.0×10^1	1.5×10^1
	2	2.2×10^6	1.3×10^1	8.0×1	7.6×10^1	—
	3	7.0×10^5	<3.6	4.4	<2.2	<2.0
	4	3.1×10^5	<5.8	1.0×10^1	1.3×10^1	1.3
	5	1.4×10^6	<3.3	9.1	2.7	1.0
	6	1.6×10^6	3.2	1.1×10^1	9.1	1.2
	7	8.9×10^4	<4.4	0.89	8.0	5.3×10^1
St. Petersburg	1	8.9×10^2	—	1.2×10^2	8.2×10^1	2.4×10^2
	2	4.7×10^7	$<3.6 \times 10^1$	2.4×10^1	5.8×10^2	—
	3	2.0×10^7	3.6×10^1	3.3×10^2	3.8×10^2	—
	4	6.4×10^2	1.0×10^2	6.4×10^2	1.5×10^2	9.1×10^1
	5	1.6×10^7	—	5.1	5.1	7.3
	6	1.4×10^7	—	1.4×10^2	3.1×10^1	7.1
	7	1.8×10^7	—	4.0×10^2	1.2×10^2	4.4×10^1
	8	5.9×10^6	5.1×10^1	5.3×10^1	$<2.7 \times 10^3$	9.3×10^1

*ND—not detected

TABLE 39. CONCENTRATION OF FECAL COLIFORM IN AEROSOL SAMPLES SPRAY SITES (cfu/mg particulate)

Site	Run	Sludge Sample (cfu/gm TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	3.2×10^5	<0.51	0.51	0.51	<0.51
	2	4.4×10^5	4.2×10^2	1.8×10^1	2.0×10^1	—
	3	1.2×10^5	<1.6	<0.99	<0.99	<0.89
	4	4.9×10^4	<2.6	0.95	1.9	<0.60
	5	1.8×10^5	<1.5	1.2	<0.41	<0.45
	6	3.2×10^5	<1.42	3.8	0.59	0.56
	7	1.5×10^4	<2.0	0.79	0.40	<0.33
St. Petersburg	1	1.8×10^5	—	3.1×10^1	2.4×10^1	1.8×10^1
	2	9.2×10^6	$<1.1 \times 10^1$	9.8×10^1	5.3×10^2	—
	3	1.2×10^7	$<1.1 \times 10^1$	1.0×10^2	9.5×10^1	—
	4	6.4×10^2	1.9×10^1	5.5×10^1	2.9×10^1	1.7×10^1
	5	2.8×10^5	—	6.6×10^{-1}	1.1	1.4
	6	2.9×10^6	—	7.3×10^1	3.5×10^1	1.8
	7	4.1×10^6	—	3.1	1.3×10^1	1.6×10^1
	8	1.3×10^5	1.9	6.1	3.1×10 1	9.0

TABLE 40. CONCENTRATION OF FECAL STREPTOCCI IN AEROSOL SAMPLES SPRAY SITES (cfu/mg particulate)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	1.4×10^5	1.2×10^1	5.3	3.8×10^1	5.8
	2	1.1×10^5	7.8	6.9×10^1	1.0×10^2	—
	3	4.5×10^4	7.1	1.1×10^1	1.3×10^1	4.0
	4	2.5×10^4	1.1×10^1	1.3×10^1	1.0×10^1	9.3
	5	1.3×10^5	3.3	3.1×10^1	1.3×10^1	1.0
	6	9.1×10^4	1.2×10^1	4.7×10^1	3.8×10^1	1.2×10^1
	7	9.3×10^3	4.4	7.1	3.6	1.5
St. Petersburg	1	4.1×10^4	—	7.1×10^1	5.3×10^1	9.6×10^1
	2	1.0×10^6	$<2.4 \times 10^1$	1.7×10^2	1.5×10^2	—
	3	7.5×10^5	$<2.4 \times 10^1$	3.3×10^2	4.0×10^1	—
	4	1.8×10^4	6.9×10^1	3.8×10^2	3.1×10^2	1.6×10^2
	5	9.7×10^4	—	8.2	3.1	7.8
	6	7.7×10^5	—	6.4	3.6×10^2	2.9×10^1
	7	8.2×10^5	—	1.1×10^2	1.4×10^2	1.1×10^2
	8	5.0×10^5	$<4.4 \times 10^1$	4.0	1.5×10^3	9.3×10^1

TABLE 41. CONCENTRATION OF MYCOBACTERIUM SPP. IN AEROSOL SAMPLES SPRAY SITES (cfu/mg particulate)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
Portland	1	8.7×10^5	1.5	1.4	4.1	ND*
	2	1.6×10^6	ND	3.6	2.3	—
	3	1.8×10^6	4.9×10^1	2.1×10^1	1.3×10^1	2.7×10^1
	4	2.8×10^7	<3.3	1.7×10^1	3.8×10^1	1.4×10^1
	5	6.0×10^6	4.0	1.1	8.9	0.60
	6	1.3×10^7	1.9	2.7	0.80	0.76
	7	4.3×10^6	2.7/<1.4	2.7	<0.53	0.04
St. Petersburg	1	3.8×10^7	—	2.4×10^1	2.4×10^1	9.6×10^1
	2	6.4×10^7	$<1.5 \times 10^1$	5.8×10^1	4.7×10^1	—
	3	6.4×10^7	2.9×10^1	4.4×10^1	5.8×10^1	—
	4	5.9×10^7	CS	CS	CS	CS+
	5	1.5×10^6	—	2.7	1.3	0.04
	6	6.1×10^7	—	1.0×10^2	1.5×10^2	4.0×10^1
	7	CS+	—	6.4×10^1	7.6×10^1	1.5×10^2
	8	CS+	1.5×10^1	7.1×10^1	9.8×10^1	1.5×10^1

*ND—not detected

+ CS—contaminated sample

TABLE 42. CONCENTRATION OF SALMONELLA IN AEROSOL SAMPLES
SPRAY SITES (cfu/mg particulate)

Site	Run	Sludge Sample (cfu/g TSS)	Sampler Position Upwind	50R	50L	100
St. Petersburg	1	2.7	—	<1.6	<1.6	8.7
	2	1.5×10^1	$<3.6 \times 10^1$	<2.9	2.9	—
	3	<7.7	IS*	<2.9	<2.9	—
	4	$<2.1 \times 10^1$	IS	<1.8	<1.8	<1.3
	5	<1.0	—	<0.09	<0.09	1.1
	6	5.5	—	$<6.7 \times 10^1$	<6.7	<1.1
	7	<5.9	—	<2.7	<2.7	<3.3
	8	4.5	<6.4	<6.9	6.9	<1.2

*IS—insufficient sample

Using the particulate data, it is possible to generate aerosolization efficiency estimates for the spray application sites. For particulate matter, equation (3) above can be written as:

$$(C_d - B)/W \cdot F \cdot D_d = E \qquad (4)$$

since impact and decay (I,λ) have no effect on particulate results. The resulting calculation of E gives the efficiency of aerosolization of the particulate or solid matter in the sludge, under the assumption that all particulates above the background (upwind) level arose from the aerosolized sludge. Diffusion estimates were generated using the formula previously given, with σ_y and σ_z being taken from "Workbook of Atmospheric Dispersion Estimates" (13). Source strength was based on the concentration of total suspended solids in the sludge sample collected for each run. Substituting these into equation (4) gives the estimated efficiencies for the two sites, and these are shown in Table 43.

There is considerable uncertainty in this estimate, but the fact that microbiological constituents are expected to congregate in the solids fraction of the sludge sample provides a basis for relating this efficiency to the microbiological concentrations.

From equation (3) we have that

$$(C_d - B)/W \cdot F \cdot D_d \cdot E = I\ e^{\lambda a_d} \quad ,$$

where all terms are shown or calculated except I and λ. Applying the logarithm transformation gives

$$\ln ((C_d - B)/W \cdot F \cdot D_d \cdot E) = \ln I + \lambda a_d$$

which is now a linear function of a_d of the form $y = a + bx$. Using a least-squares regression on the results gives estimates of $\lambda(b)$ and $I(e^a)$. These estimates of the impact factors (I) and organism decay rates (λ) can then be compared to results for sites applying wastewater in a similar manner. The I and λ values determined for the two sites are shown in Tables 44 and 45.

Virus Run Data

The special enterovirus aerosol run was conducted using nine high-volume aerosol samplers operating simultaneously. Sampling was conducted over six segments of spraying, with the collection media removed and

TABLE 43. AEROSOLIZATION EFFICIENCY ESTIMATES FOR SPRAY SITES
(percent)

Site	Run	Distance (m) 50	100
Portland	1	3.8×10^{-3}	1.4×10^{-4}
	2	4.9×10^{-4}	---
	3	2.0×10^{-4}	4.3×10^{-4}
	4	1.5×10^{-4}	5.1×10^{-4}
	5	5.5×10^{-4}	8.0×10^{-4}
	6	4.8×10^{-4}	6.1×10^{-4}
	7	2.8×10^{-4}	6.2×10^{-4}
St. Petersburg	1	1.0×10^{-3}	4.2×10^{-4}
	2	1.2×10^{-3}	---
	3	9.6×10^{-4}	---
	4	3.7×10^{-4}	4.3×10^{-4}
	5	2.6×10^{-1}	1.3×10^{-1}
	6	2.9×10^{-3}	2.2×10^{-2}
	7	1.1×10^{-3}	1.0×10^{-3}
	8	---	1.4×10^{-3}

TABLE 44. IMPACT FACTORS FOR SPRAY SITES

Site	Run	Bacteriophage	Standard Plate Count	Total Coliform	Fecal Coliform	Fecal Streptococci	Mycobacteria
Portland	1	--	--	--	--	--	--
	2	--	--	--	--	--	--
	3	155	--	--	--	--	--
	4	--	--	--	--	--	4.2
	5	23	--	517	--	--	--
	6	--	--	--	13	--	--
	7	--	82255	0.4	--	26733	--
St. Petersburg	1	73.4	0	7640	52511	307	1.4×10^{-1}
	2	--	--	--	--	--	--
	3	--	--	--	--	--	--
	4	--	--	--	--	4.7×10^{8}	--
	5	1.2×10^{8}	9.6×10^{10}	807649	3147	9.0	--
	6	335285	9823	430806	1.5×10^{7}	95894	12.7
	7	2604	2666	5354	41.2	7410	--
	8	--	--	--	--	--	--

TABLE 45. DECAY FACTORS FOR SPRAY SITES (sec^{-1})

Site	Run	Bacteriophage	Standard Plate Count	Total Coliform	Fecal Coliform	Fecal Streptococci	Mycobacteria
Portland	1	--	--	--	--	--	--
	2	--	--	--	--	--	--
	3	-0.052	--	--	--	--	--
	4	--	--	--	--	--	-0.049
	5	-0.061	--	-0.242	--	--	--
	6	--	--	--	-0.070	--	--
	7	--	-0.080	0.088	--	-0.130	--
St. Petersburg	1	0.155	1.545	0.243	0.078	0.185	0.302
	2	--	--	--	--	--	--
	3	--	--	--	--	--	--
	4	--	--	--	--	-0.072	--
	5	-1.308	-1.449	-1.021	-0.391	0.168	--
	6	-0.156	-0.014	-0.274	-0.328	-0.151	0.027
	7	0.015	0.021	-0.086	0.066	0.005	--
	8	--	--	--	--	--	--

pooled after each segment. The pooled media were concentrated in the field for shipment to the analytical laboratory, with 1500 mL of collecting medium the maximum amount that could be concentrated at one time. A total of 3542 mL was processed, which required three concentrations and three analytical samples. One hundred mL were removed for routine analysis prior to concentration. The concentrated sample for enterovirus assay represented a total of 1,470 m^3 of air, 270 m^3 per segment of each of the first five segments and 120 m^3 on the last segment where only four samplers were used. Meteorological conditions were ideal for the virus run, with 7/8 cloud cover for the first segment and 8/8 cover for the remaining segments, wind speed from 2.5 to 3.0 meters per second, and temperature at 25°C.

No human enteric viruses were detected in any of the samples sent for analysis. This represents an aerosol concentration of less than 0.0016 pfu/m^3 of air sampled. The results of the routine analyses performed are shown in Table 46 for the special virus run for both the aerosol and the sludge sample.

TABLE 46. MICROORGANISM AEROSOL CONCENTRATIONS ON ENTEROVIRUS AEROSOL RUN

Microorganism	Sludge (pfu or cfu/g TSS)	Aerosol (pfu or cfu/m^3)
Bacteriophage	8.9×10^4	3.3
Standard Plate Count	1.1×10^9	2.7×10^5
Total Coliform	2.2×10^7	6.4×10^1
Fecal Coliform	2.2×10^6	9.6
Fecal Streptococci	5.7×10^5	4.2×10^1
Mycobacterium spp.	5.8×10^7	CS*
Salmonella	$<5.8 \times 10^0$	<0.27
Enteroviruses-HeLa	0.7	<0.0016

* CS - contaminated sample.

SECTION 7

DISCUSSION

SPRAY APPLICATION OF LIQUID SLUDGE

Land application of properly digested sludges is a viable means of recycling a natural, reusable resource to the soil. There are, unfortunately, several potential hazards that can result from this practice. Some of these hazards are a buildup of toxic compounds in the soil, chemical and microbiological contamination of ground and surface waters, and the aerosol transport of pathogenic organisms. Though aerosols are generated and transported from the two methods of application studied in this grant, there are ways to lessen the impact of aerosol formation.

Most aerosol generation occurs at sites utilizing large spray guns where it is necessary to dispose of large quantities of digested sludge, usually in large, densely populated areas. Since potential exposure of large segments of the population is dependent on site location, this factor tends to become a compromise between locating the site in a sparsely populated area and keeping the costs of transporting sludge from the treatment facility to the site reasonable. Additionally, a suitable buffer zone could be established around an application site to reduce exposure of the downwind population.

When a spray gun is operated can play an important role in limiting transport of aerosols. Examples of appropriate operational conditions include limiting spraying during periods of high winds. Daytime spraying would be preferable over nighttime since periods of high solar radiation and low humidity would tend to reduce concentration of aerosols transported.

For small to medium sized publicly-owned treatment works, tank truck land application might be an adequate manner in which to utilize digested sludge. Since there are two techniques used to apply sludge from a truck, spray or subsurface injection, selection of injection equipment would eliminate direct aerosol formation. Injection also limits the amount of sludge residue remaining on the surface of the application site and the resulting potential for airborne particles of sludge to be formed from the action of surface winds on dried sludge residue.

Where injection is not used and tank truck spray application is practiced, certain precautions can be employed to limit aerosol exposure

of individuals in the vicinity of the application site. These could include creation of a buffer zone downwind of the application site during spray periods and applying only under proper meteorological conditions as at spray gun sites. As practiced at many sites utilizing either type of spray application on unvegetated soil, disking of the soil after application would alleviate the problem of airborne dried sludge residue.

Personnel performing land application at tank truck sites can limit exposure by exercising practical judgment. For example, where there is an option, direction of application would be best into the prevailing wind. Not as suitable, but still satisfactory, would be an application traverse perpendicular to the prevailing wind direction since the aerosol cloud generated would be as wide as the length of traverse. Least desirable would be applying in the same direction as the prevailing wind direction. This last mode would result in greatest exposure to aerosols of equipment operators.

MICROBIOLOGICAL AEROSOLS

Tank Truck Sites

The geometric mean concentrations of the microbiological constituents in the aerosol samples are shown in Table 47 for the two tank truck sites. From these, it can be seen that there is evidence for the generation of microbiological aerosols from the spray operation. The presence of measurable levels of fecal streptococci, especially at Longmont, is an indication that some aerosolization does occur. The problems associated with monitoring at tank truck sites reduce the sensitivity of the analyses. In each segment of the spraying at the truck sites, the samplers were downwind of the spray only when the truck was passing near the samplers. As a result, a 15-minute sampling segment might contain only 2 to 3 minutes of downwind sampling, with the balance of the collection expected to be similar to background levels. In addition, the spray is generated at a low height, and this would lead to relatively low transport of the aerosol any distance downwind. The likelihood is that there is aerosol generation and transport, but that the microbiological constituents are not transported away from the spray site to any significant extent.

Spray Sites

The geometric mean concentrations of the microbiological constituents of the aerosol samples are shown in Table 48 for the two spray sites. From these, there is a strong indication that aerosols are being generated and microbiological constituents are being transported downwind of the sprayed area. The strongest evidence for this comes from the fecal coliform and fecal streptococci data, which would be expected to be in sludge but not at measurable levels in background samples. Fecal coliform was at consistently measurable levels only at the 50-m sites downwind at Portland and were observed at an average concentration

TABLE 47. GEOMETRIC MEAN CONCENTRATION OF MICROORGANISMS-TANK TRUCK SITES

Microorganism	Site	Sample Sludge (pfu or cfu/g TSS)	Aerosol Samples (pfu or cfu/m^3) Upwind	Downwind 1	Downwind 2
Bacteriophage	Longmont	9.9×10^4	--*	--	--
	Cibolo	1.5×10^5	--	--	--
Standard Plate Count	Longmont	9.4×10^7	6.0×10^3	2.1×10^5	6.9×10^6
	Cibolo	2.7×10^8	3.8×10^4	9.8×10^5	1.2×10^6
Total Coliform	Longmont	1.5×10^6	3.8	6.0×10^2	2.7
	Cibolo	1.3×10^6	1.7	2.0×10^1	9.6
Fecal Coliform	Longmont	4.8×10^5	--	--	2.3
	Cibolo	2.0×10^5	--	1.2	1.2
Fecal Streptococci	Longmont	2.8×10^5	0.58	4.2	1.6×10^1
	Cibolo	5.3×10^4	1.7	2.7	1.2

* No mean calculated, predominance of values below detection limit.

TABLE 48. GEOMETRIC MEAN CONCENTRATION OF MICROORGANISMS-SPRAY SITES

Microorganism	Site	Sludge Sample (pfu or cfu/g TSS)	Aerosol Samples (pfu or cfu/m^3)			
			Upwind	50R	50L	100
Bacteriophage	Portland	1.4×10^4	--*	0.33	0.36	0.13
	St. Petersburg	8.1×10^4	--	1.7	1.9	0.73
Standard Plate Count	Portland	1.9×10^7	9.6×10^2	4.2×10^3	7.1×10^3	2.1×10^4
	St. Petersburg	8.0×10^7	5.1×10^4	4.9×10^4	9.3×10^4	2.4×10^5
Total Coliform	Portland	8.3×10^5	--	1.2	1.0	0.58
	St. Petersburg	1.4×10^6	0.93	2.4×10^1	2.4×10^1	1.3×10^1
Fecal Coliform	Portland	1.8×10^5	--	0.40	0.22	--
	St. Petersburg	3.9×10^5	2.0	1.0×10^1	8.7	5.4
Fecal Streptococci	Portland	5.7×10^4	0.31	2.5	2.5	0.76
	St. Petersburg	2.5×10^5	0.60	3.0×10^1	2.8×10^1	1.7×10^1
Mycobacterium spp.	Portland	4.2×10^6	0.13	0.53	0.44	0.18
	St. Petersburg	3.1×10^7	0.07	6.7	6.8	2.5

* No mean calculated, predominance of values below detection limit.

two orders of magnitude greater at the downwind than at the upwind site at St. Petersburg. Similarly, fecal streptococci were at one and two orders of magnitude greater than the background sites at the Portland and St. Petersburg sites, respectively. Elevated levels of mycobacteria were noted at the St. Petersburg site also, two orders of magnitude greater than the background levels observed. Bacteriophage and total coliform data also indicated increased levels downwind of the spray area. The inference is that elevated levels of microbiological constituents can be expected downwind of sites practicing spray gun application of liquid sludge to land.

MODELING OF AEROSOL DATA

Tank Truck Sites

The modeling of the aerosol data from the tank truck sites was limited to the calculation of relative impact factors for the various components, as discussed in Section 6. These relative impact factors can be compared to those from a site practicing spray application of wastewater by using data generated at the Pleasanton, California spray irrigation facility, which has been extensively modeled (14). The relative impact factors from the tank truck sites and those from the Pleasanton study were used to generate summary statistics and the comparisons are shown in Table 49.

As can be seen, the relative impact was noticeably lower at the sludge sites than at the wastewater spray site. The reasons for this could relate to either the matrix itself or the mode of operation or both, but the data do indicate that a different system is under study.

Spray Sites

At the spray sites, the particulate results were used to estimate aerosolization efficiencies and these used to perform some modeling of the data. The aerosolization efficiencies at Portland ranged from 0.00014% to 0.0038%, with a mean of 0.00070% and a median 0.00049%. At St. Petersburg, the range was from 0.00037% to 0.26%, with a mean of 0.037% and a median of 0.0011%. The median aerosolization efficiencies at Pleasanton, at Fort Huachuca, Arizona, and at Deer Creek Lake, Ohio were 0.33, 0.29 and 0.47%, respectively, considerably higher than those estimated in this study.

There are, however, important differences between the two types of studies. First, the previous studies dealt with spray application of wastewater, with a low percentage of solids. In those studies, a dye was added to the wastewater before spraying and was measured in the downwind samples. In this study, the efficiency was based on the collection of airborne particulate matter, under the assumption that any particulates above background were due to aerosolized solids from the study, and the actual origin of the particulate matter is unknown. Thus, these estimates have less certainty in them than those

TABLE 49. SUMMARY OF RELATIVE IMPACT FACTORS (RELATIVE TO STANDARD PLATE COUNT)

Site	Statistic	Bacteriophage	Total Coliform	Fecal Coliform	Fecal Streptococci
Truck sites (combined)	Maximum	0.17	3.6	0.12	0.21
	Minimum	3×10^{-6}	1×10^{-6}	2×10^{-6}	8×10^{-6}
	Mean	0.043	0.41	0.022	0.036
	Sample size	10	17	13	14
Pleasanton	Maximum	52	63	16	310
	Minimum	0.031	0.018	0.076	0.37
	Mean	4.2	4.9	2.6	32
	Sample size	27	29	11	21

from the wastewater spray sites where the dye could only originate in the wastewater. In addition, the aerosolization efficiencies at the sludge sites represent strictly the aerosolization of the particulate portion of the sludge, not of the combined matrix. As a result, it would be expected that the efficiency would be less on this basis and that a greater proportion of the solid fraction would fall out with distance. As a result, these efficiencies may not be unreasonable for high-volume spray application of liquid sludge and could be used for prediction of downwind concentrations of microorganisms at other similar sites.

The impact factors estimated from these studies are considerably higher than those from the Pleasanton study and the decay factors are generally comparable. Only three impact factor values fell between 0 and 1, the nominal range of that parameter, and no easy explanation is available. It most likely implies a failure of the modeling attempt to adequately describe the system due to the imprecision in obtaining estimates of efficiency indirectly and to relating the levels of microorganisms to the particulate collected by the high-volume air sampler. In addition, where no particulate results was obtained from the upwind sampler, the background was assumed to be zero, and this would affect the modeling to some extent. Since the modeling effort did not produce sufficient valid estimates of impact, no direct comparison of impact and decay factors between sludge and wastewater spray sites was warranted.

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APPENDIX A

HIGH-VOLUME AEROSOL SAMPLING PROCEDURE

SOLUTIONS:

1% Clorox
Buffers - KH_2PO_4 (71 g/L) ——————> 50 mL
Na_2HPO_4 (115 g/L) 50 mL L DI H_2O
Autoclave 50 mL of buffer in 2 oz. bottles
Add 1 mL of 5% Clorox prior to use

1% Sodium Thiosulfate
10 g Na Thio/L DI H_2O

Sterile water
Autoclave 100 mL in 4 oz. bottles prior to use

PROCEDURE:

Initial Cleanup

1. Calibrate air flow meter for 1000 lpm.

2. Disconnect electrical supply, remove side plates from unit.

3. Using Kim Wipes dipped in 100% ethyl alcohol, wipe the inside top half sides and all upper section parts. Dry with clean Kim Wipes.

4. Run disc (but not blower) and pump 1% Clorox solution through all tubes. Small amounts of the solution may be poured on the top of the disc to speed up the cleaning procedure.

5. After decontamination with Clorox solution, flush the system with the contents of a sodium thiosulfate bottle.

6. Rinse the system with the contents of a sterile water bottle. After most of the liquid has been pumped out of the system, attach a microfilter to the sampler inlet and run the blower until the disc is dry. Before all fluid has been pumped out, place 5 drops of H_2O fluid from the outlet tube on a TGY plate. Incubate at 37° for 48 hours and check for colonies.

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7. Place the ends of the tubes in a clean plastic bag and tape shut. Seal the sampler inlet and exhaust ports with aluminum foil.

Subsequent Cleanup

After the initial cleanup, Steps 4 through 8 are sufficient for future decontamination provided no contamination is indicated by the TGY plates.

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APPENDIX B

DECONTAMINATION PROCEDURE FOR LITTON MODEL M

1. After sampling sites have been selected and assigned, set up two sample tables 1 m apart. Check the tables to make sure they are level. Set an alternator off to the downwind side the full length of the extension cord and check the gas and oil levels before starting. Set the samplers in the center of each table with the control panel on the downwind side.

2. Place a piece of masking tape on a BHI media bottle and mark the level of the liquid. Run the media into the high-volume sampler and when the media returns to the bottle, mark the liquid level on the BHI bottle again. Keep the level of the liquid at this second mark throughout the run by adding sterile water as is necessary. Keep the BHI bottle covered with its lid and masking tape during the run.

3. Make sure that the plate is wetting properly and then set the liquid flow rate at 3-6 mL/min. Adjust the air flow rate to read the calibrated 1000 lpm mark.

4. After 3 minutes of BHI media recirculation, turn the high-voltage power supply to 14 kV and sample for a minimum of two of the 12- to 15-minute sludge spraying periods. Instructions will be given for starting and stopping sampler operation. Pay careful attention to the liquid levels of the BHI and to excessive arcing.

5. At the end of the run period, turn off the high-voltage power supply and pull the supply line out of the BHI reservoir. Run the liquid pump and the air blower until all the liquid has been collected in the original bottle. Make the level up to the original 100 mL mark on the bottle by pumping sterile water through the sampler.

6. Clean up the samplers either in the field or the lab as instructed.

HIGH-VOLUME PARTICULATE SAMPLING PROCEDURE

1. After removing filter frame center a numbered preweighed glass fiber filter (filter number to the outside) on the back screen of a high-volume sampler. Replace frame and hand tighten wing nuts by hand.

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2. Locate sampler on the furtherest downwind side of the Model M sampler in a horizontal position. Locate the sampler so that there are no obstructions in front of the filter. Power this sampler on a separate extension cord directly from the alternator.

3. At the start of the first BHI recirculation turn on the blower. Read the rotameter after 5 minutes and record the value. Record time.

4. Continue to run sampler until last run segment has been completed. Note rotameter value and time. After turning off blower, carefully remove filter and place in filter bag.

APPENDIX C

DESCRIPTION OF LITTON MODEL M HIGH-VOLUME AEROSOL SAMPLER

"The Model M Sampler is designed to continuously collect particulate matter from a large volumetric flow rate of air (approximately 1000 liters/minute) and deposit it into a small amount of liquid (flow rate of 2mL/min). This effects a volumetric concentration factor on the order of 5×10^5. Basically, the sampler is an electrostatic precipitator of a rather unusual configuration. With reference to the schematic diagram, Figure C-1, and an interior view, Figure C-2, aerosol is drawn into the unit through a converging nozzle and passes through the center of the high-voltage plate. It then flows radially between this plate and a lower rotating collection disc. An electric potential of 15,000 volts, which is maintained across a 11/16-inch spacing between the plate and disc, creates two effects: (1) A corona is emitted from a ring of 60 needles that is located concentric to the air inlet. Particles, exposed to air ions created from the corona, acquire an electrical charge. (2) The electric field provides the driving force to precipitate charged particles onto the lower disc.

"Liquid is pumped onto the center of the collection disc and, because of the centrifugal force, forms a thin moving film over the entire disc surface. Particles collected on the film are transported to a rotating collection ring where the liquid is removed by the pickup. Subsequently, the liquid drips into the collection funnel where it is pumped to a receiver located outside the sampler.

"To accommodate a broad range of sampling situations, several variable features are incorporated into the unit. These are:

Air Flow Rate	400 to 1200 liters/minute
Liquid Flow Rate	0 to 8mL/minute
Disc Speed	0 to 45 rpm
High Voltage	0 to 20 kilovolts

"When the sampler is in operation, the air flow rate is read directly from a calibrated meter on the front panel and is adjusted with a blower control potentiometer (see Figure C-3). Both disc speed and pump flow rate are controlled by high and low range toggle switches, together with potentiometers. Although no direct readouts are provided for these two variables, calibrations are easily obtained so the arbitrary scales on the potentiometers can be converted to actual

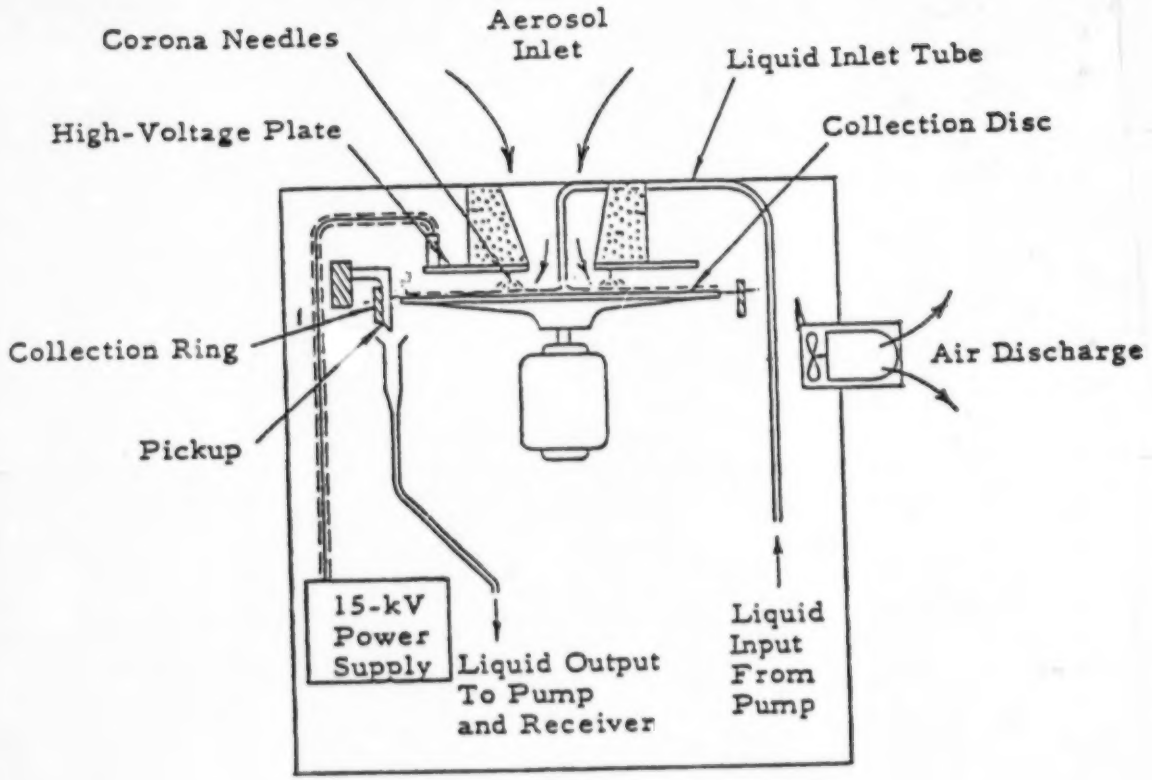

Figure C-1. Schematic Diagram of Large-Volume Air Sampler System

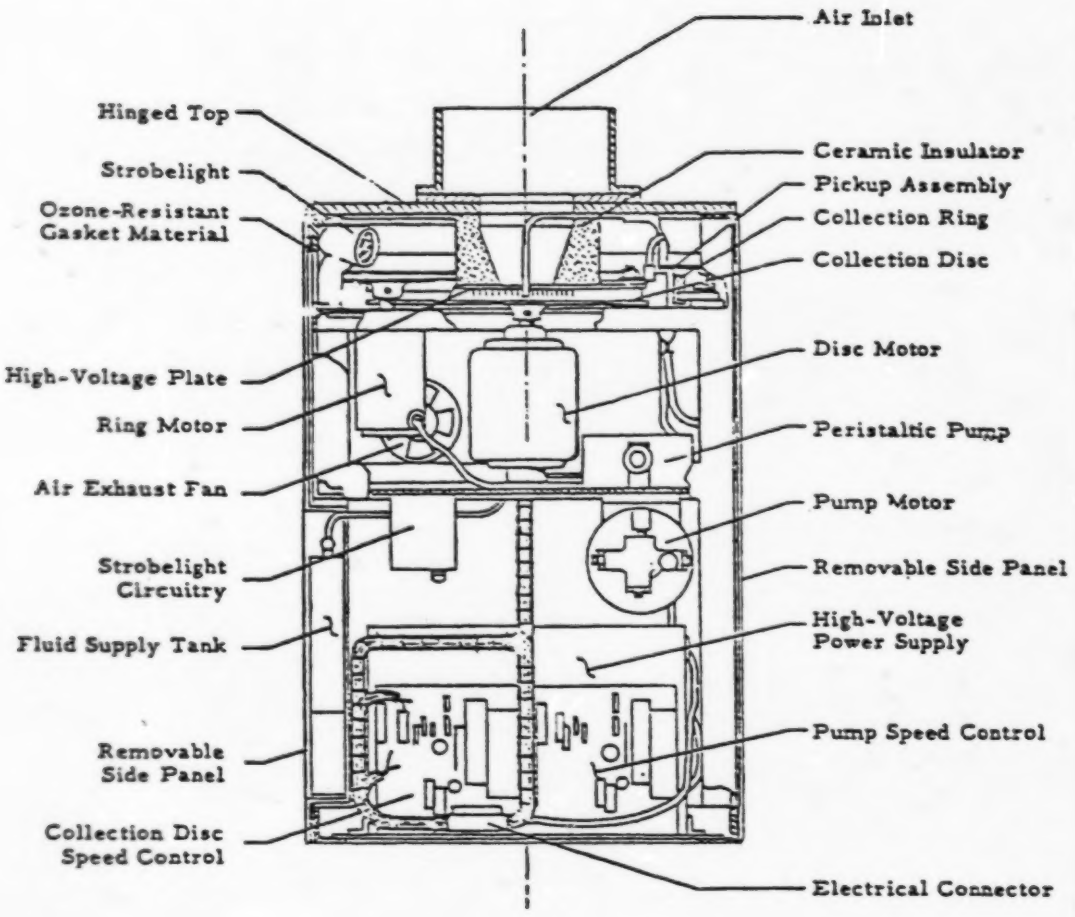

Figure C-2. Interior View of Large-Volume Air Sampler

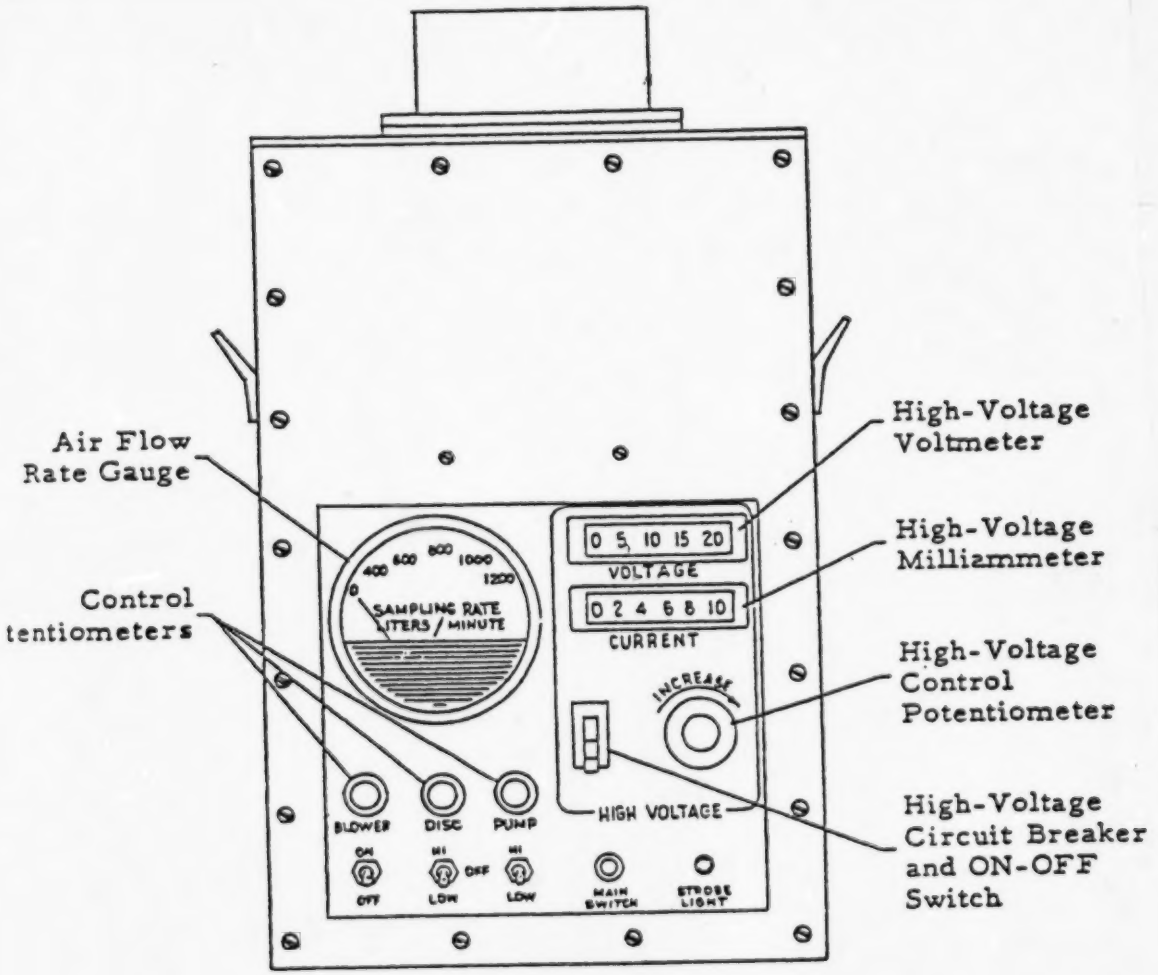

Figure C-3. Instrument Panel of Model M Large-Volume Air Sampler

speed or flow rates. The high-voltage system is set with the aid of a potentiometer and is provided with the meter to show voltage and current."[1]

To facilitate visual observation of the surface condition of the disc in operation, the operator made observations through the windows with the aid of a flashlight. The air flow rate was set at 1000 liters/minute.

[1] Litton Model M Large-Volume Air Sampler: Instruction Manual, Report No. 3028. Minneapolis, Minnesota (1966).